DOES NOT CIRCULATE

UNIVERSITY OF MICHIGAN
MAY 7 1958
MEDICAL LIBRARY

БЮЛЛЕТЕНЬ
ЭКСПЕРИМЕНТАЛЬНОЙ
БИОЛОГИИ
И МЕДИЦИНЫ

Bulletin of EXPERIMENTAL BIOLOGY AND MEDICINE

VOL. XLIII, No. 2

February, 1957

IN ENGLISH TRANSLATION

CONSULTANTS BUREAU, INC.

227 WEST 17TH STREET NEW YORK 11, N. Y.

an agency for the interpretation of international knowledge

ADVANCE MONTHLY, TABLES OF CONTENTS - IN ENGLISH

Of All The Soviet Journals Now Being Translated

An Express Translation Service — Anticipate your needs

Avoid Duplication of research for only $25.00 per year

12 issues, on annual subscription

Each month Consultants Bureau will publish English translations of Tables of Contents of issues of Soviet journals published in the Soviet Union two months previously. These will be mailed to subscribers in a convenient, attractive booklet immediately on publication.

This booklet will include Tables of Contents of all Soviet journals being translated and published on a regular basis by Consultants Bureau, by other firms, by learned societies, etc.; i.e., *all Soviet journals being translated into English on a continuing basis.* As new journal translations are begun, their Tables of Contents will be included. See right hand column for journals which will be included from the very beginning of this service.

For your convenience in anticipating translation needs, each Table of Contents will cite: *The estimated date of publication of that issue in English* (when this information is available from the publisher); the name and address of the organization from which the translation may be obtained; the yearly subscription price; and the price of individual issues, or papers (when these are sold separately).

The first issue of EXPRESS CONTENTS will be published in May 1958 and will include all January and February material received by Consultants Bureau by March 30, 1958. (The June issue will contain March–April material. The July issue, May material, etc.) As a means of bringing you up-to-date on the vast amount of significant material recently published in the Soviet Union, we also make this offer: Tables of Contents of *all the 1957 issues of these same journals,* in one volume, to be published in June 1958. Each Table of Contents in this volume will carry the same information described above; many issues will already have been published in translation, and will be so marked. This important volume is only $15.00.

Bull. Exp. Biol. & Med.
Biophysics
Sechenov J. Physiol.
Probl. Oncology
Probl. Virology
J. Microbiol., Epidemiol., Immunobiol.
Probl. Hematol. and Bl. Trans.
Microbiology
Plant Physiology
Doklady: Botan. Sci.
Doklady: Biol. Sci.
Biochemistry
Doklady: Biochemistry
Pharmacol. and Toxicol.
Colloid Journal
Bull. Acad. Sci., Div. Chem. Sci.
J. Gen. Chem.
J. Anal. Chem.
J. Appl. Chem.
Doklady: Chem. Sections: Chem.; Agrochem.; Geochem.; Chem. Tech.
Doklady: Phys. Chem.
Doklady: Geol. Sci.
Phys. of Met. and Metalog.
Metallurgist
Metal Sci. and Treatment
J. Tech. Phys.
Acoustics
Doklady: Physics
Bull. Acad. Sci., Div. Phys. Sci.
Doklady: Appl. Phys.
J. Exp. and Theoret. Phys.
Soviet J. of Atomic Energy
Automation and Remote Control
Radio Engineering
Radio Eng. and Electronics
Electrical Communication

★ ★ ★

ANNUAL SUBSCRIPTION PRICES:

Subscriptions cover all calendar year issues of the original Russian journals

1 Copy	$25.00 each
10–100 Copies	18.00 each
100–500 Copies	15.00 each

500 & above, $11.50 each and free of charge: your own special organizational cover—write for details and sample.

1 Volume: **1957 CONTENTS** $15.00

SPECIAL INTRODUCTORY OFFER:
One copy of the first issue (May 1958, January–February 1958 Contents) of EXPRESS CONTENTS, only $6.00, This purchase price will be credited against the price of subscriptions received by June 30, 1958.

Write for free EXPRESS CONTENTS BROCHURE, and fully descriptive catalogs of our current Russian translation-publishing program, including journals, monographs, symposia, conferences, dictionaries, etc.

CONSULTANTS BUREAU, INC.

227 WEST 17th STREET, NEW YORK 11, N. Y. — U.S.A.

Telephone: ALgonquin 5-0713 • Cable Address: CONBUREAU, NEW YORK

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

(BYULLETEN EKSPERIMENTALNOI BIOLOGY I MEDITSINY)

Volume XLIII No. 2

February, 1957

Editorial Board

V. N. Chernigovsky (Editor)
A. Ya. Alymov, G. N. Kryzhanovsky (Secretary)
A. V. Lebedinsky (Acting Editor), I. N. Maisky, V. A. Negovsky, V. A. Sanotsky,
L. G. Trofimov, M. A. Vorontsova, O. E. Vyasov, N. A. Yudaev, G. N. Silov

A Publication of the Academy of Medical Sciences of the USSR

IN ENGLISH TRANSLATION

Copyright, 1958

CONSULTANTS BUREAU, INC.

227 West 17th Street

New York 11, N.Y.

The National Institutes of Health of the Public Health Service has supported in part the cost of publishing BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE through the purchase of copies for distribution to American medical libraries.

Printed in the United States

Annual Subscription $20.00

Single issue 10.00

Note: The sale of photostatic copies of any portion of this copyright translation is expressly prohibited by the copyright owners. A complete copy of any paper in this issue may be purchased from the publisher for $7.50.

THE DOMINANT FOCUS OF EXCITATION IN THE FORMATION OF A CONDITIONED REFLEX

M. Ya. Rabinovich and L. G. Trofimov

Electrophysiologic Laboratory (Head – Professor L. G. Trofimov), Institute of the Brain
(Director – Full Member of the USSR Academy of Medical Sciences, Professor S. A. Sarkisov)

(Received April 27, 1956. Presented by Full Member of the
USSR Academy of Medical Sciences, S. A. Sarkisov)

As the result of studies of the mechanisms of cortical function by means of electroencephalography made in recent years some new facts have emerged concerning the relation between a dominant focus of excitation in the cortex and the mechanism of temporary connection formation. I. P. Pavlov [6] suggested that the formation of a conditioned reflex could be based on the ability of an adequately excited cortical focus to direct towards itself weaker stimuli arriving from the outer and inner environments; this concept brought together the mechanism of the formation of temporary connections and the properties of a dominant focus.

Electrophysiologic facts now available indicate the existence of a dominant focus of excitation in the cortex during the formation of a conditioned reflex. There is, however, not a single opinion on the location of this focus. Some workers [1, 7, 8] consider that the dominant focus arises, as suggested by Pavlov, in the cortical representation of unconditioned reflex activity. According to the data of others [4, 10] the formation of a dominant focus occurs at that point of the cortex on which the stimulating signals impinge.

The present work reports certain data obtained during the study of electrical phenomena in the cortex of the motor, auditory and visual analysors during the formation of a motor-defense conditioned reflex to sound and light; these data are relevant to the question of a dominant focus in the formation of temporary connections.

EXPERIMENTAL METHODS

Experiments were carried out on dogs with implanted electrodes according to the method usually employed in this laboratory [2]. A conditioned motor-defense reflex (flexion of the hind limb) was established in the animals to rhythmic auditory and visual stimuli which were reinforced by electric stimulation of the hind limb skin with a lag of 3-4 seconds.

Records were taken from the cortical terminations of the motor (g. cruciatus post.), auditory (g. ectosylvius med.) and visual (g. lateralis and g. ectolateralis) analysors on the hemisphere contralateral to the limb being stimulated.

DISCUSSION OF RESULTS

An analysis of the electroencephalograms (EEG) recorded during the formation of a conditioned reflex (present and previous studies [3]) shows even at the beginning of the formation of a temporary connection the conditioned stimulus evoked a definite increase in the amplitude and frequency of potentials in all the areas investigated – motor, auditory and visual. These changes appear with the first few combinations of the conditioned and unconditioned stimuli and precede the appearance of the conditioned motor reaction. Similar generalized changes of potentials towards excitation are noted in the period when the stimulus signal begins to elicit a definite motor reaction.

Later, as the conditioned reflex becomes more firmly established, the stimulus signal evokes changes of potential predominantly in those cortical zones between which a temporary connection is being formed. Figure 1 shows that definite increases of the amplitude and frequency of potentials in the cortical terminations of the motor and auditory analysors are seen when a sufficiently firm motor-defense reflex to sound had been established (144 combinations). The amplitudes in the motor cortex increase from 10-20 μV in the "background" record to 35 μV at the moment when the signal stimulus begins to act and up to 35-55 μV at the moment associated with the conditioned motor reaction. In the case of the auditory cortex the amplitudes increase from 20 to 40 μV at the moment of the sound stimulus and up to 60 μV during the motor reaction. The frequency rises from 35-36 to 40 per second in the motor cortex and from 25-27 to 32 per second in the auditory cortex. The first period of enhanced amplitude and frequency recorded from the motor and auditory cortex at the moment when the signal stimulus begins to act has a small latent period and lasts about 0.5 seconds.

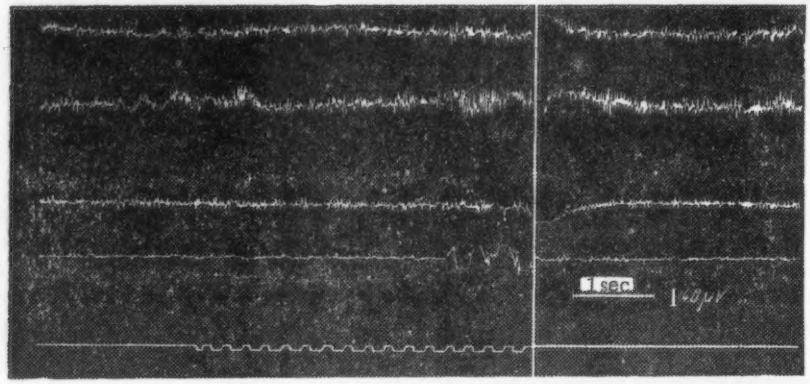


Fig. 1. Electrical activity of the cortical terminations of the motor, auditory and visual analysors during a conditioned defense reflex to sound. Dog Zhuk. Right hemisphere. Bipolar record.
Records from above downward: cortical electrogram of motor analysor; same, auditory analysor same, visual analysor; record of movement of the hind leg. Signal marker of the auditory stimulus (sound 500 cps with frequency interval 4 per second). Artefact from electric stimulation of skin excluded from all records.

In other words, the cortical centers of the analysors directly involved are in a state of excitation which, in the present case, begins almost simultaneously with the action of the signal stimulus, becomes stronger at the moment of the motor reaction and ends after the discharging event—movement. No changes of this nature are observed in the potentials of the visual cortex which, in this particular case, is not directly involved in the formation of the temporary connection. In fact, during the formation of the conditioned defensive reflex to sound the amplitude of the waves on the visual cortex was diminished even in the "background" records.

If the establishment of a firm conditioned defensive reflex to sound is followed by the development of a defensive reflex to light, the changes in the cortical motor and auditory analysors during the action of the new signal stimulus remain the same in character as during the action of sound. Figure 2 shows records of the electrical activity of the motor, auditory and visual cortex in the same dog but using a light signal stimulus started 5 minutes after the 140th combination with sound. The potentials in the motor cortex showed an increase in amplitude from 15-20 μV in the "background" record to 40-45 μV under the action of the light stimulus, while the frequency rose from 35-38 to 44-45 cycles per second. In the cortical auditory analysor the amplitudes increased from 20 to 50-60 μV and the frequencies from 26-28 to 35-38 cycles per second.

In the cortical visual analysor on which the signal stimulus impinged directly there was barely perceptible increase in the amplitude of the waves, while the frequencies remained the same as in the "background" record. Judging by the character of the EEG changes, a state of excitation is produced in the motor and auditory

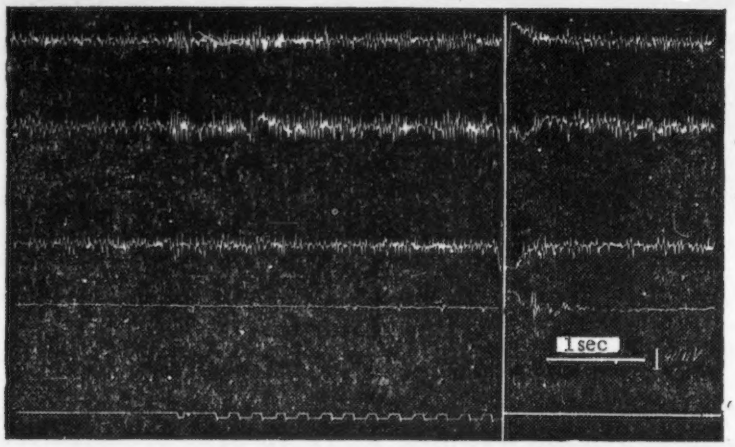


Fig. 2. Changes in the electrical activity of cortical motor, auditory and visual analysors under the action of light signal stimulation. Dog. Zhuk. Right hemisphere. Bipolar recording.
Records from above downward: four upper records same as in Fig. 1. Lower record – signal marker for conditioned light stimulus (electric lamp with flicker frequency 4 per second).

analysors, evoked by impulses from other cortical areas, and this focus of excitation can inhibit the responses from other cortical areas to stimulation directed to them. When a conditioned reflex reaction to light is established, then an analogous state of excitation to that described for the association of the motor and auditory analysors arises in the motor and visual areas.

An interesting fact was noted in the course of the experiments. If a random movement occurred in the animal's hind leg (the limb under investigation) unconnected with any of the stimuli being used, changes in the EEG potentials could be detected which depended on the particular conditioned stimulus which preceded the random movement. If, for example, the random movement occurred directly after the conditioned reflex response to sound (Fig. 3,a) the EEG changes were similar to those which took place during the conditioned reflex to sound, i.e., the motor and auditory areas showed increased amplitudes and frequency of the waves, while the changes in the visual area were much less pronounced. If, however, the random movement occurred following the conditioned reflex reaction to light (Fig. 3,b) excitation took place in the cortical components of the motor and visual analysors, while the cortical components of the auditory analysor showed signs of active inhibition – appearance of slow waves.

The facts described demonstrate that when a conditioned reflex is formed, a focus of excitation appears in the cortex; this focus has a number of distinctive features. It appears every time under the action of a conditional stimulus and disappears after the discharging act – the conditioned reflex reaction. The focus of excitation, arising under the action of the signal stimulus, embodies both cortical areas involved in the association and inhibits the third area which is not directly concerned in the formation of a given temporary connection (see Fig. 1). The same focus of excitation can arise in response to impulses from other sources and it then reduces the ability of other cortical areas to respond to stimuli aimed directly at them (Fig. 2). And, finally, this focus of excitation possesses certain inertia as shown by the fact that random movements of the limb under investigation can reproduce the distinctive EEG changes which preceded these random movements (see Fig. 3).

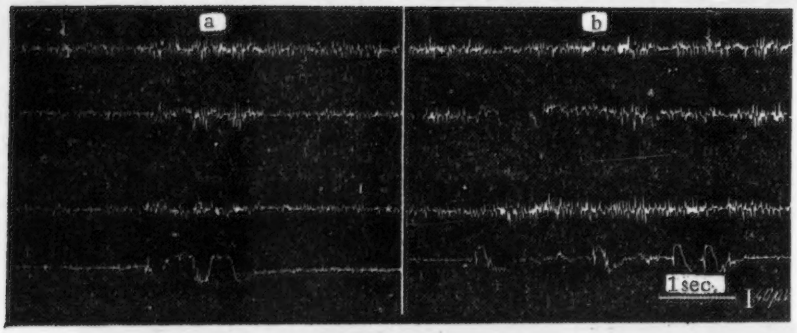


Fig. 3. Changes in the electrical activity of cortical motor, auditory and visual analysors during random movements of the hind leg being studied, after the use of conditional stimuli.
a) auditory stimulus; b) (right) light stimulus. Dog Zhuk. Right hemisphere. Bipolar record. Records from above downward same as in Fig. 1.

These distinctive features of the focus of excitation which appear during the formation of a conditioned reflex show that the focus has some properties of dominance. As can be seen from the encephalograms a dominant focus is not formed at some one point on the cortex but extends to and includes those cortical structures between which the connection is being established. The EEG s show that all the changes undergone by this focus under the influence of various signal stimuli characterize it as a functional entity; its structural components, however, can be different, and often widely separated, areas of the cortex. This is in complete agreement with the views of A. A. Ukhtomsky, who defined dominance as a phenomenon based on the excitation of a whole constellation of centers.

Cases where the action of a new signal stimulus reproduces cortical changes associated with a state of excitation characteristic for the preceding temporary connection but does not elicit the conditioned reflex reaction merit particular attention (see Fig. 2). It would seem that the excitation of the cortical components of the motor and auditory analysors clearly reflected in the EEG should evoke a corresponding motor reaction. The absence of such reaction can be explained by the new signal stimulus restoring only part of the previous dominance. A. A. Ukhtomsky [9] supposed that cortical and somatic components should be distinguished in the dominant entity, and that in connection with this "the preceding dominance is restored either in a very contracted way with very small inertia—only by the cerebral components, or with the full previous inertia, occupying the centers for a long time and displacing from them other reactions." Since under the influence of a new signal stimulus—light—the previous dominance expressed itself only in excitation of the motor and auditory areas of the cortex the new signal stimulus evidently restored only the cortical components "with barely perceptible excitation in the muscles" [9]. The somatic components of the previous dominance (motor reaction) remained inhibited.

Application of electrophysiologic methods to the study of conditioned reflex activity in animals has revealed that the electrographic evidence of excitation of the corresponding cortical areas is not always associated with a motor reaction even in those cases when an effective and firmly linked signal stimulus is used.

On the other hand, it is not infrequently observed that a conditioned motor reaction may be very definite and pronounced with no discernible changes (or only very small changes) in the EEG from the corresponding areas (Fig. 4). Such "unsuccessful" records are encountered particularly frequently when the conditioned reflex is firmly established, and investigators tend to disregard them and omit them from demonstrations. Yet they could be taken as evidence of the possibility of restoring only the somatic components of the dominance with "barely discernible" participation of the cortical components. These findings coincide with the observations of L. A. Novikova, V. S. Rusinov, and A. F. Semiokhina [5]. The latter were studying cortical association (or connection) under the influence of an artificially induced (direct current) dominant focus and found that in some cases the stimulus elicited movement of the limb without perceptible change in the electrical activity of the cortex, while in other cases stimulation produced definite changes in the EEG without reflex movement of the limb.

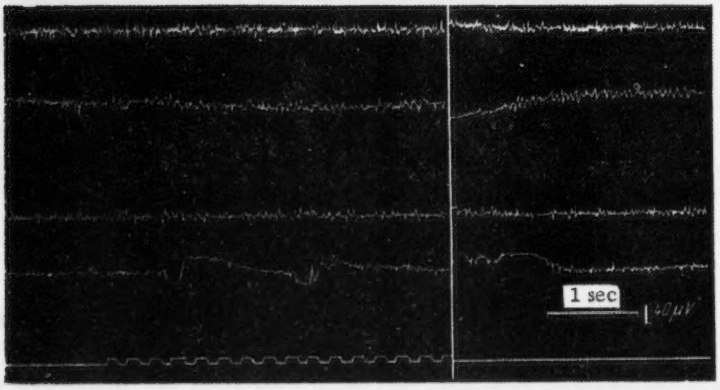


Fig. 4. Electrical activity of cortical components of the motor, auditory and visual analysors during a conditioned defense reflex to sound. 136th combination. Dog Zhuk.
Records from above downward same as in Fig.

The present authors realize that the facts submitted are far from solving this important neurophysiologic problem. The aim of this work was not so much the solution as the re-posing of the question of interrelations between the properties of dominance and physiologic mechanisms of the formation of temporary connections.

SUMMARY

Electroencephalographic data on the formation of a dominant focus of excitation during the development of defense conditioned reflexes provoked by light or sound were analyzed. The experiments were performed on dogs with permanently implanted electrodes.

It has been established that during the development of conditioned reflex connections the formation of a focus of excitation of the dominant type is taking place in the cerebral cortex. The dominant focus incloses cortical structures involved in the formation of the conditioned reflex bonds.

LITERATURE CITED

[1] M. N. Livanov, in: 50 years of I. P. Pavlov's Teachings,* p. 248-261 (1952).

[2] R. N. Lurie and L. G. Trofimov, Fiziol. Zhur. SSSR 42, p. 348-356 (1956).

[3] R. N. Lurie, M. Ya. Rabinovich, and L. G. Trofimov, Zhur. Vysshei Nerv. Deyatel 6, 863-871 (1956).

[4] R. M. Meshchersky in: Proceedings of the Institute of Higher Nervous Activity, Physiology Section, 1, p. 265-278 (1955).

[5] L. A. Novikova, V. S. Rusinov, and A. F. Semiokhina, Zhur. Vysshei Nerv. Deyatel 6, 844-861 (1952).

[6] I. P. Pavlov, Collected Works,* Moscow Leningrad, 3, Book 1, 63 (1951).

[7] G. T. Sakhnulina, Doklady Akad. Nauk SSSR 104, No. 1, 153-156 (1955).

[8] G. T. Sakhnulina, Doklady Akad. Nauk SSSR 104, No. 2, 332-334 (1955).

[9] A. A. Ukhtomsky, Collected Works,* Leningrad, Vol. 1, p. 200 (1950).

[10] A. Angyan, and T. Hasznos, Acta Physiol. Acad. Sci. Hung., 1951.

* In Russian.

IONO-HUMORAL INTERRELATIONS IN THE PROCESS OF APPEARANCE AND DEVELOPMENT OF VAGAL INHIBITION OF THE HEART

COMMUNICATION I

THE INFLUENCE OF ATROPINE ON THE NORMAL AND DESYMPATHIZED FROG HEART

I. A. Keder-Stepanova

Electrophysiologic Laboratory of the S. P. Botkin Hospital, Moscow

(Received December 29, 1954. Submitted by Full Member of the Academy of Medical Sciences of the USSR, M. S. Vovsi)

The present series of communications is devoted to descriptions of experimental data indicating certain distinctive features of the ionic, humoral and polarization factors and their interrelations in the process of the appearance and development of vagal inhibition of the heart.

In the course of development of various concepts of the mechanism of vagal inhibition much attention was paid to the fact that the usual effect disappeared when the vagus was stimulated against the background of atropine action. Loewi and Navratil [5] established the possibility of differential elimination of the vagal effect. They showed that under the influence of atropine vagal stimulation was accompanied by liberation of acetylcholine (i.e., from the humoral point of view the nerve endings were not touched) but the inhibitory effect was absent (consequently acetylcholine did not exert the usual effect).

It was later established that under the influence of atropine not only was there a change in the cardiac reaction to acetylcholine but there was also impairment of the potassium ion activity in the process of development of the vagal effect [4]. Thus the action of atropine disturbs the usual interrelation of the ionic and humoral factors and this, evidently, leads to the disappearance of vagal inhibition.

The present author considers that a study of the altered activity of potassium ions and of acetylcholine as well as of the polarization properties of the atropinized heart should lead to proper understanding of the interrelations of these factors in the process of the development of inhibition of the normal organ.

It was essential to check the action of atropine on heart muscle. Special attention was paid to the effect of atropine on the polarization properties of the heart as no data on the subject could be found in the literature.

EXPERIMENTAL

Experiments were performed on isolated frog hearts (Rana temporaria) using the Samoilov method and that of "circular" perfusion. Using the first method the heart, with a Straub cannula inserted into the arch of the aorta, was left attached to the frog's head by means of dissected vago-sympathetic trunks. The second method permitted the direct observation of the effect exerted by the substance being tested on the venous sinus of the heart.

The isolated heart preparation, connected to the frog's head by vago-sympathetic trunks, was set up; a small funnel was inserted into the inferior vena cava, the end of the funnel being drawn out to form a cannula; a second cannula was inserted into the arch of the aorta and connected to a narrow bent tube. The funnel was filled with Ringer's solution or with the test substance in Ringer's solution. The perfusion fluid left by way of the

aortic cannula, passed through the tube and re-entered the venous cannula (funnel). The amount of fluid in the whole system did not exceed 2-3 ml. Ventricular contractions or contractions of both the atria and the ventricle were recorded on a moving smoked drum. The cardiac action was recorded electrographically by means of an Einthoven string galvanometer (large Engelman model) connected to a two-stage balanced direct current amplifier. The input resistance reached $6 \cdot 10^6$ ohms, the output—5000 ohms. The maximal sensitivity (outflow current) was 45 mA/v; amplification (direct current) approximately $3 \cdot 10^8$. The use of directly coupled d.c. amplifier converted the electrographic string galvanometer apparatus into a potentiometric one. Thus, changes in the potential of the heart muscle were recorded and not changes in resistance (an error permitted in some investigations of the polarization properties of the heart muscle). In all the experiments with electrographic recording monophasic potentials were led off by the M. G. Udelnov method. The exploring electrode was placed on an intact area of the active heart. The indifferent electrode, consisting of a loop of a fine cotton-wool wick soaked in Ringer's solution, was tightened round the top of the heart, isolated by a tight ligature and cauterized.

The electrodes used were the nonpolarizable Dubois-Raymond type.

Part of the experiments were performed on frogs with chronically desympathized hearts. The frogs were anesthesized with ether. The anesthesized frog was placed on its back, the lower jaw was pulled down with Pean's forceps and a small incision was made in the mucosa of the upper jaw along the midline, posterior to the eyes. The mucosa was drawn apart and by means of blunt dissection with glass hooks the head ganglion was dissected as well as part of the sympathetic trunk leading to it from the stellate ganglion. The head ganglion was completely and bilaterally extirpated. No hemorrhage accompanied the operation; the oral mucosa was sutured and painted with iodine. According to B. I. Lavrentiev 8-10 days after bilateral extirpation of the head sympathetic ganglion regeneration of post-ganglionic sympathetic fibers can be observed in the frog heart. The operated frogs in the present experiments were kept on ice in an aquarium for 25-35 days; control frogs were kept together with the operated ones.

Atropine, varying in concentration from 10^{-3} to 10^{-6}, was prepared with Ringer's solution from standard 0.5% solution of atropine sulphate or from the solid preparation.

The experimental data given in the present communication were obtained in 1947-1949.

RESULTS

In the first series of experiments more than 300 tests of the action of atropine on isolated frog hearts were carried out under different experimental conditions. Figure 1 shows the most typical effects of atropine on the strength and rhythm of cardiac contractions. In 80% of the cases the introduction of atropine into the heart was followed by a positive effect, but the strength of contractions returned to normal within 20-40 minutes. The inotropic effect reached 20-25% on the average, its magnitude being independent of the concentration of atropine used. Thus, Fig. 1, a) shows the effect of atropine in 10^{-5} concentration, while Fig. 1, b that of a 10^{-4} concentration; in the first case the positive inotropic effect is 30%, in the second case 28%, and sometimes the interrelation of the effects is reversed.

A negative inotropic effect during the action of atropine was observed in approximately 14% of cases; Fig. 1, c) shows that a concentration of 10^{-4} evokes such an effect reaching 21%, while a concentration of 10^{-5} (Fig. 1, d) decreases the strength of contractions by 20%. It must be stressed again that the effects obtained are independent of the concentration of atropine used (concentrations of 10^{-3} and 10^{-6} were also used). A positive chronotropic effect was observed in 36% of the cases; it reached, on an average, 10%. A biphasic effect of atropine on the rhythmic activity of the heart was noted in 19% of the cases; the cardiac rhythm, first slowed and then accelerated by atropine, returned to normal within 30 minutes. In the remaining cases no change in the rhythm of cardiac contractions occurred.

The data obtained make it possible to assert that the positive inotropic effect which is seen in 80% of the cases immediately after the introduction of atropine can be one of the characteristic features of its physiologic action on the heart. Haberlandt [3] who observed positive and chronotropic effects in the heart of Ranae esculentae following the introduction of atropine (concentration 10^{-3}) suggested that these effects were the consequence of excitation of sympathetic nerve endings in the heart muscle by atropine. He did not observe any positive atropine effects after regeneration of cardiac nerves following section of vago-sympathetic trunks.

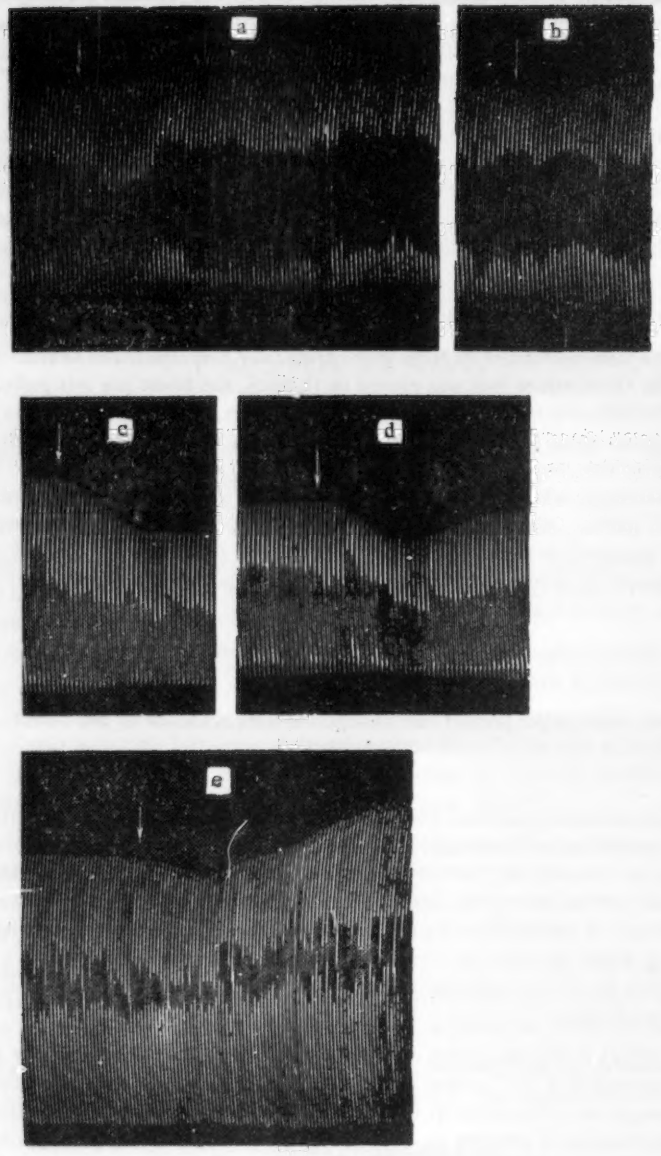


Fig. 1. Mechanograms of isolated frog heart perfused with Ringer's solution. The arrow indicates introduction of atropine, replacing Ringer's solution. e) effect of atropine on desympathized heart (frog operated on February 20th, experiment performed March 26, 1948).

The work of Haberlandt is difficult to assess since he does not give illustrations of his experiments. However, taking his findings into account, investigation of the action of atropine on desympathized frog hearts was undertaken. Forty-one of fifty-two desympathized hearts gave a definite positive inotropic effect with atropine concentrations of 10^{-4} and 10^{-5}. The effect reached 25% on the average. Figure 1, e) gives the kymogram of an experiment which shows that the strength of contraction increased by 23% (the operated frog was kept from February 20 to March 25). Under these conditions a positive chronotropic effect was demonstrated in approximately 30% of the cases. Therefore, sympathetic nerve endings do not participate in the increased contractions of the heart observed under the influence of atropine. The present author considers that this series of experiments suggests that the magnitude of the positive effects evidently depend on the initial functional state of the heart. Moreover, the positive effects of atropine action are most marked during the spring and early summer and in the autumn.

In the second series of experiments 56 tests of the action of atropine on the resting potential and monophasic action potentials of the isolated heart were carried out. In all cases some phenomena were observed which indicated certain distinctive features of the physiologic action of atropine. M. G. Udelnov [1, 2], investigating polarization phenomena occurring in the heart under various conditions (in particular during vagal stimulation) established that the positivity displayed by the heart muscle was accompanied by a growth of the amplitude of the monophasic action potential; negativity produced a decrease in the amplitude of the monophasic action potential. Electrograms recorded from the heart (Fig. 2) show that the introduction of atropine is accompanied by a small increase in resting potential (9% in Fig. 2,a) and 11% in Fig. 2,b); the same records show the increase in the amplitude of the monophasic action potential immediately after the introduction of the active substance. However, 1 minute (Fig. 2,a) or $1\frac{1}{2}$ minutes (Fig. 2, b) later the amplitude of the monophasic action potential returned to normal, despite the persistently raised resting potential. In 7 cases the resting potential was decreased immediately after the introduction of atropine with a parallel decrease in the amplitude of the monophasic action potential, but in the course of the experiment the resting potential increased and the amplitude returned to the original value.

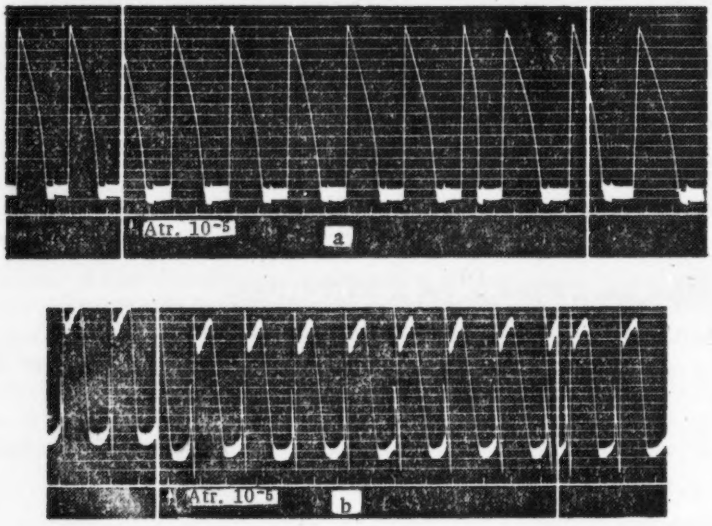


Fig. 2. Electrograms of isolated frog heart. Arrow indicates moment of replacement of Ringer's solution by atropine. Shift of the base line downwards indicates increase of resting potential.

The action of atropine on the heart was accompanied by an increase in the resting potential, which could take place phasically, the end result being expressed in the development of positivity in the heart muscle without a parallel change in the amplitude of the monophasic action potential.

The latter fact makes it possible to assume the existence of a stabilizing effect of atropine. The present author considers that atropine, by inducing positivity in the heart muscle and in some cases producing a phasic effect, creates conditions under which a change of potential exceeding its active level is impossible.

There are some indirect data in the literature which would support such an inference. Thus, Wedd and Blair [8] who investigated the action of atropine on the electrogram of turtle heart, showed that this substance lowered the conductivity of the heart muscle when the heart rate was very high. No changes occur when the heart rate is normal. Rice and Ross [7] note that the normal potential of the intestine reaches 40-100 mv, the surface of the mucosa being negative with respect to the serous layer. If this potential changes under the influence of various factors atropine restores it quickly to the normal level. Rehm and Hokin [6] also showed that when the rate of secretion and the magnitude of the potential of the intestinal wall underwent a change the action of atropine was to reduce secretion to zero and restore the normal value of the potential.

Differences in the action of atropine on the strength and rhythm of contraction of individual hearts at different times of the year, the return of positive changes of strength and rhythm to normal in the course of experiment, as well as the fact that the resting potential of the cardiac muscle increases without a parallel increase in the amplitude of the action potential, all suggest the possibility that atropine can stabilize the bio-electric tonus of the cardiac muscle. The results of experiments performed on desympathized hearts demonstrate the direct action of atropine on heart muscle; the observed positive effects do not result from excitation of sympathetic nerve endings.

SUMMARY

The effect of atropine on the strength and frequency of contractions, rest and action potentials of the normal and desympathized hearts of frogs was studied. Atropine acts directly upon the cardiac muscle, causing a slight increase in the frequency and strength of the contractions. At the same time the resting potential of the heart muscle is increased with no parallel rise in the amplitude of monophasic action potentials. The data obtained suggest the possibility that atropine stabilizes the bioelectrical tonus of the cardiac muscle.

LITERATURE CITED

[1] M. G. Udelnov, Proceedings of the 7th All-Union Congress of Physiologists, Biochemists, Pharmacologists, Moscow, p. 313-316 (1947).

[2] Ibid, in the book: Mater Experimental and Clinical Electrocardiography,* Moscow, p. 129-138 (1953).

[3] L. Haberlandt, Zschr. f. Biol., Bd. 80, S. 137-142 (1924).

[4] E. Lehnarz, J. Physiol., v. 86, p. 37-38 (1936).

[5] O. Loewi, and E. Navratil, Arch. ges. physiol. Bd. 206, S. 123-134 (1924).

[6] W. Rehm and L. Hokin, Am. J. Physiol. v. 149, p. 162-176 (1947).

[7] H. V. Rice and R. T. Ross, Am. J. Physiol. v. 149, p. 77-94 (1947).

[8] A. Wedd and H. A. Blair, Proc. Soc. Exptl. Biol. a. Med., v. 60, p. 64-66 (1945).

*In Russian.

CEREBRAL CIRCULATION UNDER REFLEX INFLUENCES

COMMUNICATION I

CEREBRAL BLOOD SUPPLY ON STIMULATION OF SMALL INTESTINE INTEROCEPTORS

N. M. Ryzhova

Laboratory of Respiration and Circulation Physiology and Pathology (Head – Professor M. E. Marshak) of the Institute of Normal and Pathologic Physiology (Director – Full Member of the Academy of Medical Sciences of the USSR, Professor V. N. Chernigovsky), Moscow

(Received January 14, 1956. Submitted by Full Member of the Academy of Medical Sciences of the USSR, V. N. Chernigovsky

Studies conducted at this laboratory have established that the lumen of cerebral vessels alters in opposite directions when hypertension is elicited in two different ways – by hypothalamic stimulation and by compression of the carotid arteries. Stimulation of the hypothalamic area produced cerebral hyperaemia and dilatation of cerebral vessels, while decreased impulses from the carotid sinus baroceptors (compression of the carotid arteries) was accompanied by decreased cerebral blood supply and constriction of cerebral blood vessels. Analysis of such differences in the blood supply to the brain led to the conclusion that the different vascular reactions associated with the same increase in arterial pressure must indicate the participation of different mechanisms. As the experiments showed, the increased tonus of cerebral vessels observed on compression of the carotid arteries was determined by the decrease in impulses from the carotid sinus baroceptors. Increased cerebral blood supply accompanying centrally induced hypertension, on the other hand, was determined by the combination of raised arterial pressure with diminished tonus of the cerebral vessels caused by an increase in impulses from the carotid sinus baroceptors. The present work was undertaken to make further analysis of factors affecting the tonus of cerebral vessels possible; a study was made of cerebral blood supply in hypertension induced by stimulation of interoceptors in a segment of the small intestine.

A great number of communications have appeared in the last decade dealing with the effect of interoceptor stimulation on arterial blood pressure and respiration (V. N. Chernigovsky and collaborators [3, 4]). The work of Meyer and Pribram [6] concerning the effect of gastric interoceptor stimulation on the pial vessels is well known. There are several investigations [1, 2, 5, 7, 8, 9] devoted to the study of the lumen of pial vessels on stimulation of sensory nerves (femoral and sciatic). Some of the workers found constriction while others found dilatation of the cerebral vessels, the inconsistency being explained by changes in arterial blood pressure level and in respiration.

EXPERIMENTAL

Experiments were performed on 34 adult dogs weighing between 6 and 10 kg. Operation was carried out under morphine-urethane anesthesia. Changes in the rate of blood flow in the dura and pia were determined by the thermoelectric method and were taken as an indicator of cerebral blood supply. In three experiments the rate of blood flow was recorded from the hypothalamic area. A trephine opening was made in the parietal, occipital or temporal bone and special flat thermoelectrodes, constructed in the laboratory, were placed on the dura. The thinness of the dura permits the assumption that the thermoelectrode would pick up changes in the rate of blood flow both in the dura and the underlying pia. Needle thermoelectrodes (Jibbs type) were used for

the determination of the rate of blood flow in the hypothalamic area; they were introduced through the trephine opening at the intersection of the coronary and sagittal sutures. The needle thermoelectrodes were inserted through the dura to a depth of 32-34 mm.

Arterial blood pressure was recorded by means of a mercury manometer from the femoral artery; respiratory movements were recorded by a pneumograph connected to a Marey's tambour. Rate of cerebral blood flow, arterial pressure and respiratory movements were recorded synchronously on a photokymograph. Stimulation of the interoceptors was produced by inflation of a loop of small intestine within the abdominal cavity with air under 60-120 mm (of mercury) pressure. The most effective stimulation in these experiments proved to be that obtained with 80 mm pressure. Repeated stimulations were applied at intervals of not less than 15 minutes.

RESULTS

In 32 (of 34) experiments inflation of a loop of small intestine was seen to be accompanied by a rise of arterial blood pressure by 10-15 mm of mercury (in different experiments). The rise in pressure usually started 2-3 seconds after the beginning of inflation continuing to rise until the end of stimulation or, having reached a maximum within 15-20 seconds, beginning to fall but never reaching the original level while inflation was in progress. On cessation of inflation the return of arterial pressure to the initial level was delayed in most cases: the pressure remained raised for 2-3 minutes or longer. Only in 3 experiments did the arterial pressure reach the initial level in the course of the first minute after cessation of inflation. The changes in cerebral blood flow were not stereotyped. In 22 experiments increased rate of blood flow was observed. In 19 of these experiments the rate of blood flow increased during stimulation synchronously with the rise in arterial pressure and on cessation of stimulation the return of arterial pressure and blood flow to the original level occurred parallel.

The synchronous increase in the rate of cerebral blood flow and in arterial pressure does not provide information as to the state of the cerebral blood vessel lumen, since it can depend on raised pressure within the vessels (increased rate of blood flow and passive stretching of the vessel wall) as well as on active stretching of vessels. However, in 3 experiments the inter-relations of changes in cerebral blood flow and in arterial pressure were such as to suggest active cerebral vasodilatation associated with stimulation of the intestinal receptors. The kymogram of one of these experiments is given in the Figure, (a). In this experiment the rate of blood flow in the meninges and in the hypothalamic area during inflation of the small intestine increased as the arterial pressure rose. On cessation of stimulation the arterial pressure remained at the raised level, reached at the end of stimulation, for 40 seconds, after which it slowly began to fall. The rate of blood flow, however, continued to increase, reaching a maximum after 30 seconds, and then began to decrease slowly.

These experimental findings suggest that the increased cerebral blood supply during interoceptor stimulation is ensured not only by passive stretching of the vessels under the influence of increased pressure within the vessels, but also by an active decrease of the tonus of cerebral vessels (as the result of nervous or humoral factors).

Changes of the rate of cerebral blood flow observed in the remaining 10 experiments also indicate that interoceptor stimulation leads to changes in the tonus of cerebral blood vessels. Thus in 5 of these experiments a diphasic reaction of the rate of blood flow was seen: an initial rise followed by a decrease. Part (d) of the illustration shows a kymogram of an experiment in which the rate of blood flow began to drop after an initial rise (over 20 seconds). On cessation of stimulation the rate of blood flow continued to drop below the initial value against the background of still raised arterial pressure; only after 28 seconds gradual restoration of blood flow occurred as the arterial pressure decreased. In another case (b) the initial rise was followed by a drop of both the rate of blood flow and of arterial pressure, but the blood flow began to decrease earlier and fell markedly below the initial level towards the end of stimulation. Its decrease continued after the end of stimulation for a further 15 seconds, after which it gradually returned to the original value. Arterial pressure, however, returned to pre-stimulation level 15 seconds after cessation of stimulation, followed by a small rise.

A small but definite decrease in the rate of blood flow was observed throughout the period of stimulation against a background of relatively little increased arterial pressure in an experiment represented by the kymogram in part (c) of the illustration. Further confirmation of the presence of factors increasing the tonus of cerebral vessels during stimulation of intestinal interoceptors is furnished by the data of the following four experiments. In these experiments no change in the rate of blood flow occurred against a background of pronounced rise in

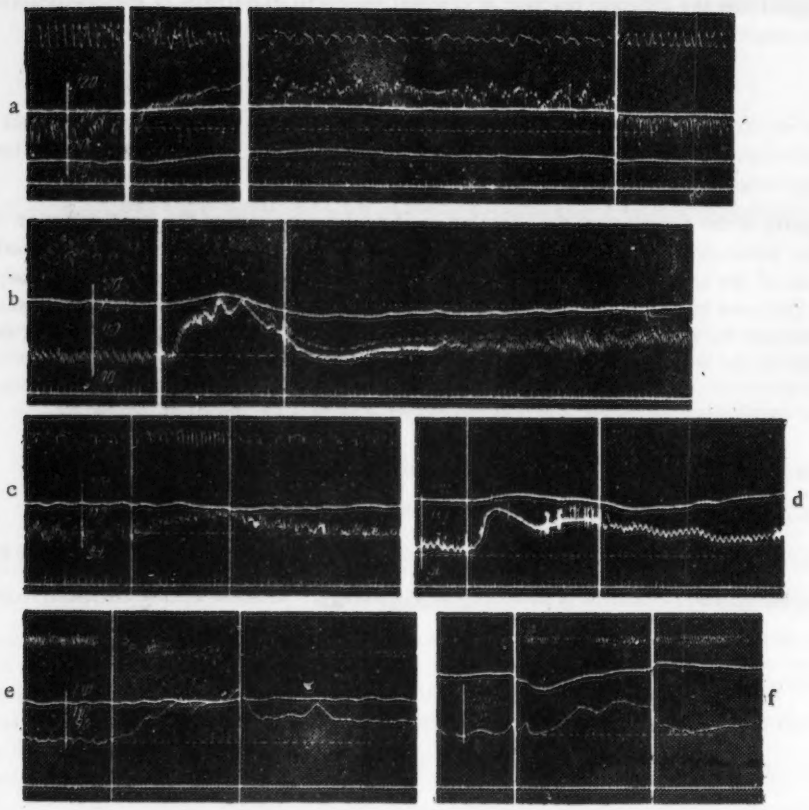


Fig. Reaction to inflation of a loop of small intestine in different animals. Records from above downward: a) respiratory movements, rate of blood flow in hypothalamic area, arterial pressure, rate of blood flow in dura and pia, time marker; b), d) rate of blood flow in dura and pia, arterial pressure, time marker; c), e), f) respiratory movements, rate of blood flow in dura and pia, arterial pressure, time marker (seconds).
Time marker) 2 seconds. Dotted line—level of factors under investigation before stimulation; vertical lines—start and end of stimulation.

arterial pressure (e), which is only possible with constriction of cerebral blood vessels. The absence of changes in the rate of cerebral blood flow could not be due to inertness of the thermoelectrode, since considerable changes in cerebral blood flow were recorded in the same experiments on compression of the carotid arteries (f).

These experimental results lead to the conclusion that the nature of the changes in cerebral blood supply can depend on the inter-relation of vasodilatory and vasoconstricting influences elicited by interoceptor stimulation. In cases where a decrease in tonus is associated with increased arterial pressure, an increase of blood supply results. When the tonus is increased the raised arterial pressure may not overcome it and in these cases the cerebral blood supply either remains unchanged or decreases on interoceptor stimulation.

The diversity of reaction to interoceptor stimulation in different animals can depend on: 1) the functional state of the vasomotor center and 2) the tonus of cerebral vessels at the moment of stimulation. The fact that a stereotyped reaction of arterial pressure—a rise—was observed in all the experiments speaks against the first

factor. Moreover, no changes were seen in the arterial pressure reaction to carotid pressor reflex in different animals.

It would appear that the different reaction of cerebral vessels to stimulation of the interoceptors of the small intestine is determined chiefly by the initial tonus of the vessels.

SUMMARY

Changes of the blood velocity in the dura and pia mater of the brain and in the hypothalamus region in the case of the inflation of the small intestine loop have been analyzed by the application of the thermoelectrical method on 34 dogs under morphine-urethane anesthesia.

In the majority of the experiments the stimulation of the interoceptors of the small intestine brings about the increase of the blood circulation in the brain with synchronous rise of the arterial pressure; in other cases under the same rise of the arterial pressure the decrease of blood circulation, or a biphasic response, i.e., the original increase followed by the decrease of blood circulation is observed. Such different responses of the blood vessels of the brain and the interoceptors being stimulated is connected with the different tonus of the blood vessels of the brain at the moment of stimulation.

LITERATURE CITED

[1] V. Dybkovsky, Voenno-Med. Zhur. 87, No. 5, 48-53 (1863).

[2] E. I. Eselevich, Byull. Eksptl. Biol. i Med. 20, No. 6, 22-24 (1945).

[3] V. N. Chernigovsky, Transactions of the Army-Navy Medical Academy, Leningrad, 17, 395-422 (1949).

[4] V. N. Chernigovsky, Problems of Interoceptor Physiology,* Moscow-Leningrad 1, 551-558 (1952).

[5] W. Jacobi and G. Magnus, Arch. Psychiatr., Bd. 74, S. 126-138 (1925).

[6] M. Monnier and E. Streiff, Arch. ges. Physiol. Bd. 243, S. 478-484 (1940).

[7] O. Muller and R. Siebeck, Ztschr. Eksptl. Pathol. Therap., Bd. 4, S. 57-88 (1907).

[8] H. Nohtnagel, Arch. f. pathol. Anat. Bd. 40, S. 203-213 (1867).

* In Russian.

GASTRIC MECHANORECEPTOR REFLEXES BEFORE AND AFTER HEMIDECORTICATION

S. S. Musyashchikova

Receptor Physiology Laboratory (Head – Full Member of the Academy of Medical Sciences of the USSR, Professor V. N. Chernigovsky) and the I. P. Pavlov Laboratory of Pathologic Physiology (Head – Professor V. S. Galkin), the I. P. Pavlov Institute of Physiology (Director – Academician K.M. Bykov) of the Academy of Medical Sciences of the USSR, Leningrad

(Received May 21, 1956. Submitted by Full Member of the Academy of Medical Sciences of the USSR, V. N. Chernigovsky)

The present work was undertaken with a view to elucidate the role of the cerebral cortex in the unconditioned reflex activity of interoceptors.

The work of V. N. Chernigovsky [13] and collaborators [3, 5, 8] has shown that interoceptor reflexes to circulation are greatly enhanced in decerebrate animals. Respiratory reflexes are inhibited by decerebration (section at the inter-collicular level). It has been established that the cerebral cortex exerts a regulatory effect on visceral reflexes to changes in circulation and respiration [4] and in skeletal musculature [2, 6]. According to the data in the works cited, the regulatory action of the cortex is, in the case of some visceral reflexes stimulating, in others – inhibitory. In critical experiments the inhibitory effect of the cortex is mainly observed.

It was decided to investigate the changes in interoceptor reflexes in a series of experiments, using the method of partial decortication.

Photo-recording of cutaneous galvanic reflexes to gastric distention was used as a test of reflex activity. The cutaneous galvanic reflex is widely used both clinically and experimentally in man as an index of vegetative reactions. But only a small number of works [7, 11, 12] have been devoted to the subject in the case of warm-blooded animals.

EXPERIMENTAL

Experiments were performed on cats with gastric fistulas. The skin potentials were led off in two ways: silver electrodes were placed on the soft foot-pads of the hind limbs; one electrode was placed on the foot-pad, the other on the skin of the hind leg (shin). A piece of cotton wool soaked in physiologic solution was placed between the electrode and the animal's skin. The cutaneous potentials were recorded by a mirror galvanometer (type M-21). The galvanometer readings were recorded on photosensitive paper on an electrokymograph. The potential differences were measured in millivolts (made possible by using a compensatory device attached to the galvanometer).

The animals were familiarized with the experimental conditions over a period of several days. They were laid down on a mat, and the hind legs were fixed by an assistant, since movements affected the changes of potential. Half an hour before the beginning of the experiment a rubber balloon was inserted into the stomach through the fistula; the balloon was connected with a rubber bulb and a mercury manometer. The skin galvanic reflexes to gastric distension were elicited by inflating the balloon. Reflexes were recorded at intra-gastric pressures of 40, 60 and 80 mm of mercury. The stimuli were applied at 5-7 minute intervals.

RESULTS

Skin galvanic reflex responses to gastric distention in cats with intact nervous system.

The investigation of skin galvanic responses in cats is complicated by the fact that the potential differences of the skin are unstable and inconstant. Therefore, the original potential level in the record appears not as a straight line as in the case of man and dogs, but as an undulating curve. The components of this curve reach 1-5 mv. These peculiarities complicate the course of cutaneous galvanic reactions in cats and the analysis of reflex activity is thus made more difficult.

The reflex response of the skin to gastric distention was most frequently expressed by increased negativity of the foot-pad with respect to the shin skin potential, which was reflected by a downward deflection of the galvanometer with respect to the base line (Fig. 1, c). The reverse effect was also observed in response to stimulation (Fig. 1, a and b). The reactions were different in different animals; three types of response could be distinguished: 1) gastric stimulation was accompanied by a single change in potential, recorded as a deflection from the initial level (Fig. 1, a); 2) the cutaneous galvanic reflex appeared as fluctuations of skin potentials with greater amplitude and frequency than those observed in the original background (Fig. 1, b); 3) the cutaneous galvanic response appeared both as changes in the potential differences and simultaneous increase in amplitude and frequency of oscillations as compared to the original background (Fig. 1, c).

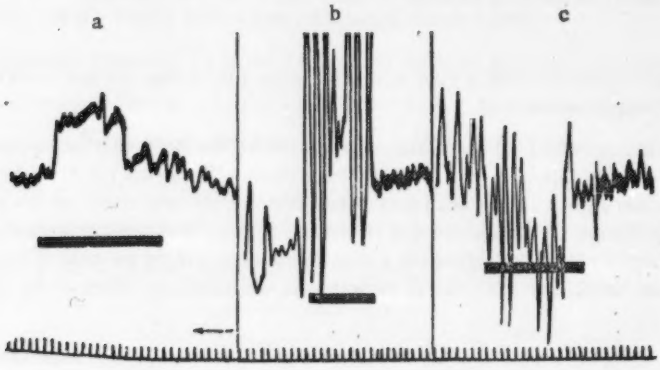


Fig. 1. Cutaneous galvanic response to gastric distention (pressure 60 mm of mercury).
a) cat Dymka, b) cat Belaya mordochka; c) cat Murzik. Records from above down: skin potentials, stimulus marker, time marker 5 seconds.

The type of reaction in a given animal was constant throughout a series of experiments. A response could usually be observed at balloon pressure of 40 mm of mercury. The response increased with growing distention of the stomach. This was observed with all types of cutaneous galvanic reactions. In those experiments where it was of the second type increased stimulation led to increased enhancement of the amplitude and frequency of the oscillations as compared to the background.

Partial limitation of the receptor field was used in an attempt to find the source of variability of the reflex response to gastric distention This was achieved by painting the gastric mucosa with a 2-3% solution of cocaine. Under these conditions it was found that the skin galvanic reaction did not disappear. Only in one of 18 experiments did the reaction fail to appear even in response to strong stimulation (pressure 80 mm of mercury). In all the other cases, the responses were definite but their magnitude was somewhat less than in the normal. In some experiments the threshold was raised after anesthesia and no response was seen to pressure of 40 mm of mercury. As can be seen from Figure 2, when the gastric mucosa was painted with cocaine, the amplitude of the potential difference oscillations decreased sharply, the background was smoothed out and the

reaction followed the third type of response. Regardless of the type of response prior to anesthesia the reflexes after anesthesia consisted of a shift in the potential differences from the original level and of small oscillations.

Analysis of the results of this series of experiments provides grounds for supposing that the decreased reflex reactions and increased thresholds are connected with elimination of gastric mechanoreceptors. When cocaine is introduced into the stomach it first of all affects the receptor endings situated in the mucosa, but on penetrating further it can also affect the mechanoreceptors of the muscle and serous layers. As the result the magnitude of the reaction decreases and the threshold rises, while at the same time the fluctuations of potential difference in the original background are considerably diminished and the reflex response is made up of a simultaneous shift of potential difference from the original level and its fluctuation.

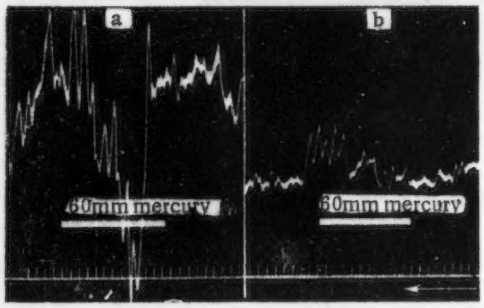


Fig. 2. Cutaneous galvanic reflex response to gastric distention (pressure 60 mm of mercury) after partial elimination of gastric receptors.
Cat Krasivy.
a) before anesthesia of stomach; b) after anesthetising stomach with cocaine. Record from above down same as Fig. 1.

Cutaneous galvanic reflex responses to gastric distention in cats after hemidecortication.

The reactions described above were studied in cats with intact cortex over a period of 3-4 months. Sixteen to thirty-five experiments were carried out on each animal prior to operation. When the background reactions had thus been determined the cats were subjected to extirpation of the cortex from the left cerebral hemisphere. The cortex was completely removed from the occipital, parietal, most of the temporal and frontal areas. The basal portion of temporal cortex was spared. Five animals were used.

The behavior of the animals post-operatively corresponded to descriptions reported in the literature [1, 9, 10]. Marked incoordination of movements was noted in the first few days after operation. The right hind limb was weak and did not participate actively in locomotion. In response to calling or the sound of dishes indicating feeding time, the operated cats performed rotary movements from right to left round themselves prior to approaching the source of the sound. With time the phenomena described subsided and after 2 months, it was difficult to distinguish on cursory examination the operated animals from healthy ones.

The cutaneous galvanic reactions to gastric distention were tested from the 7th post-operative day onward. The results of the experiments showed that 7-10 days after operation the reflexes under investigation were depressed. They appeared only in response to strong stimulation (pressure 80 mm of mercury) and their magnitude was diminished compared to the normal. Weaker stimuli (pressure 40-60 mm of mercury) elicited no response. Gradual restoration of the reflexes and lowering of the thresholds were observed during subsequent days. After 3 weeks or a month, their magnitude exceeded that observed prior to operation.

As can be seen from Figure 3, one month post-operatively the reflexes had increased. The threshold had become lower; if prior to operation the animal showed no response to gastric distention equivalent to 40 mm of mercury (Fig. 3, a) one month after operation the same strength of stimulation elicited quite a definite change in cutaneous potential difference (Fig. 3, b).

The reflexes in operated animals were kept under observation for $2^1/_2$ years. The reflexes became normal after 6-8 months, their magnitude not exceeding the original value. Figure 3, c represents an experiment performed 7 months post-operatively.

The background galvanogram was preserved after operation. The potential differences were inconstant in the same way as pre-operatively.

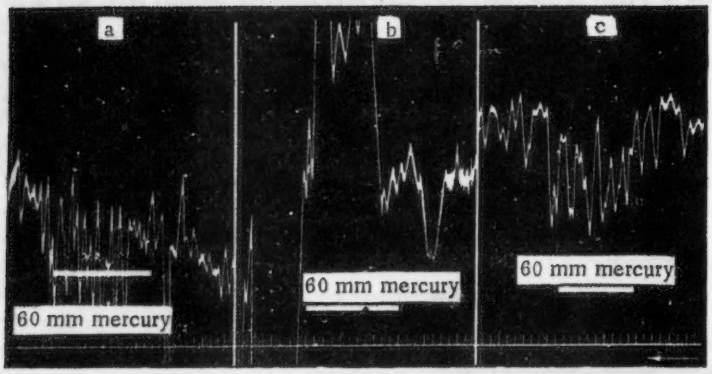


Fig. 3. Cutaneous galvanic reflex responses to gastric distention (pressure 60 mm of mercury) before and after extirpation of cortex. Cat.
a) before operation; b) one month after extirpation of cortex from the left hemisphere; c) 7 months after operation. Records from above down the same as in Fig. 1.

DISCUSSION OF RESULTS

The material obtained indicates that hemidecortication leads to changes in interoceptive reflexes to stimulation of the gastric mechanoreceptors. Seven to ten days post-operatively the reflexes were depressed; their magnitude was small and the threshold high. Later on they increased and exceeded the initial background.

The first phase of the observed changes can be explained by the inhibition of reflexes seen in the early post-operative period being due to extensive and gross cerebral trauma. As the healing proceeds the reflexes are restored and subsequently (after a month) exceed the original. It can thus be concluded that under normal conditions the cerebral cortex inhibits the interoceptive skin galvanic reflex under investigation. If the fact that decerebration is associated with increased interoceptive reflexes, as shown by other indicators, is taken into account, the observed inhibitory effect of the cerebral cortex on the interoceptive reflex under investigation becomes a particular case of cortical inhibition of many unconditioned reflexes.

Further restoration of the reflex to the original values evidently occurs with the help of the compensatory mechanisms of the intact cerebral hemisphere.

SUMMARY

Galvanic skin reflexes to the distention of the stomach were studied on cats before and after hemidecortication.

From 7 to 10 days after extirpation, reflexes were depressed owing to the trauma after-effect. They increased sharply after a month, the thresholds having dropped. From 6 to 8 months after extirpation, reflexes attained the original level. Increase of the reflexes under study in the experimental animals may be due to the partial elimination of the inhibitory effects of the cerebral cortex. Restoration of the reflexes is due to the compensatory mechanisms of the intact hemisphere.

LITERATURE CITED

[1] E. A. Astratyan, Fiziol. Zhur. SSSR 24, No. 1-2, 36-41 (1938).

[2] I. A. Bulygin, Physiologic Problems of Interoception,* Moscow-Leningrad, No. 1, 91-114 (1952).

[3] G. A. Kovaleva, Byull. Eksptl. Biol. i Med. 27, No. 6, 415-418 (1949)..

[4] G. A. Kovaleva, Physiologic Problems of Interoception,* Moscow-Leningrad No. 1, 255-264 (1952).

[5] V. A. Lebedeva, as cited by V. N. Chernigovsky, Trudy VMMA, XVII, 395 (1949).

[6] O. S. Merkulova, Physiologic Problems of Interoception,* Moscow-Leningrad No. 1, 369-381 (1952).

[7] S. S. Musyashchikova, Fiziol. Zhur. SSSR, 37, No. 6, 718-726 (1951).

[8] S. S. Musyashchikova, Byull. Eksptl. Biol. i Med. 37, No. 1, 13-18 (1954).

[9] M. A. Pankratov, Bull. Lesgaft Scientific Research Institute, 41, No. 1-2, 251-267(1938).

[10] N. F. Popov, Studies of the Physiology of the Cerebral Cortex in Animals,* Moscow (1953).

[11] L. N. Stelmakh, XVI Conference on the Problems of Higher Nervous Activity, Moscow-Leningrad 207 (1953).

[12] V. I. Filistovich, Byull. Eksptl. Biol. i Med. 27, No. 3, 177-180 (1949).

[13] V. N. Chernigovsky, The Afferent Systems from Viscera,* Kirov (1943).

* In Russian

THE ROLE OF THE PANCREAS IN THE COMPLEX REFLEX REGULATION OF CARBOHYDRATE METABOLISM

I. S. Kanfor

From the Laboratory of Gas and Heat Exchange Physiology (Prof. R. P. Olnyanskaya, Chief) of the Department of General Physiology (Prof. A. V. Rickel, Chairman) of the Institute of Experimental Medicine of the Academy of Medical Sciences of the USSR, Leningrad

(Received January 2, 1956. Presented by K.M. Bykov, Member of the Academy)

In previous work [3, 4, 6] we showed that the act of eating exerts a complex reflex influence on the process of carbohydrate metabolism. The present work is devoted to the question of the role of the pancreas in the complex reflex regulation of carbohydrate metabolism in connection with food intake.

E. and L. Hedon [13] found that $2\frac{1}{2}$ hours after total pancreatectomy an increase begins in the blood sugar content. N. S. Veller, P. M. Charnaya and B. M. Rodkina [1, 2] performed this operation on dogs and obtained diabetic hyperglycemia 4-7 hours after the animals awoke from anesthesia. These authors have shown that, against a background of depression of the central nervous system with amytal narcosis, diabetic hyperglycemia is detected in dogs 12-30 hours after depancreatization, arising only against a background of the restoration of the function of the central nervous system.

The examinations cited were performed in such a way that an irreversible disturbance to the organism as a whole ensued, due to extirpation of the pancreas; this is their chief drawback.

In critical experiments on dogs and cats Grafe and Meythaller [12] and also T. Kosako [14] established that the injection of glucose solutions into the femoral and portal veins produces severe hyperglycemia, while injection into an artery of the pancreas produced only negligible hyperglycemia, and at times even hypoglycemia. By using the angiostomic method and inserting a cannula into the pancreatico-duodenal vein, N. P. Kotchnewa and E. C. London [7-11] established a small reduction in the sugar content of the blood flowing from the pancreas. In the opinion of these authors the pancreas secretes its hormone periodically in response to an excess of sugar in the blood, while epinephrine enters the blood continuously. To all appearances the incretion of insulin on an empty stomach does not occur.

EXPERIMENTAL METHODS AND RESULTS

We employed a new method in order to decide the question of the role of the pancreas in the regulation of carbohydrate metabolism in the organism as a whole, namely the temporary exclusion of the pancreas from the general blood stream. For this end, the pancreatico-duodenal vein was placed in a specially constructed cannula [5]; by turning the screw of the cannula, we temporarily stopped the entrance into the general blood stream of blood flowing from the pancreas.

Critical experiments were performed on cats. Blood samples were taken simultaneously from a vein of the pancreas and from the peripheral blood. Their sugar content was determined both during normal blood circulation and after exclusion of the blood of the pancreas from the general blood stream.

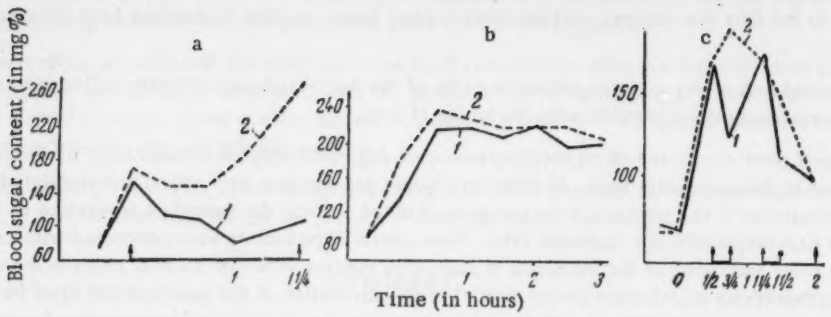


Fig. 1. Blood sugar content.
a) during exclusion of the pancreas from the general blood stream; b) during normal blood circulation in the pancreas; c) during short-term exclusion of the pancreas from the general blood stream and its subsequent inclusion; 1) blood sugar content of the pancreatic vein; 2) the same in the general blood stream; ↑↑)Flow of blood halted.

The results of the first series of experiments, which were performed on 6 cats (Fig. 1,a), showed that after exclusion of the pancreas from the general blood stream the sugar content of the peripheral blood increased, while that of the blood of the pancreatic vein decreased.

In the second series of experiments, performed on 3 cats, an examination was made of the sugar content of the blood flowing from the pancreas and that of the peripheral blood during undisturbed blood circulation through the pancreas and during partial exclusion of the pancreas from the general circulation. Under these circumstances there occurred a simultaneous and almost identical increase in the sugar content of the blood of the pancreatic vein and of the general blood stream (Fig. 1, b).

Thus it is apparent from the experiments that under the normal conditions of existence of an organism the pancreas is just that organ in which there takes place a considerable absorption of sugar from the circulating blood. Our data differ from the results of the experiments of E. C. London and N. P. Kotchnewa [10] who noted only a negligible absorption of sugar from the blood by the pancreas.

In order to make more precise the question as to how reversible are the changes which arise in the regulation of blood sugar content, experiments were conducted, the purpose of which consisted in clarifying the period necessary for restoration of normal sugar content in the blood of the pancreatic vein and in the peripheral blood after temporary exclusion of the pancreas from the general circulation.

In experiments performed on 6 cats the pancreas was excluded from the general blood stream for 15 minutes, and then blood circulation was restored once again in the pancreas for 30 minutes. Exclusion of the pancreas from the general blood stream produced in only 15 minutes a considerable reduction in the sugar content of the blood of the pancreatic vein and, simultaneously, an increase in the sugar content of the peripheral blood. When the pancreas was subsequently re-included in the general blood stream, within 30 minutes the sugar content of the blood of the pancreas and that of the general blood stream once again became almost identical (Fig. 1, c).

The data put forward indicate a direct relationship between sugar content, both that of the general blood stream and that of the blood flowing from the pancreas, and the conditions of blood circulation in the pancreas itself; they permit one to assume that uninterrupted circulation of blood through the pancreas is necessary for the maintenance of the normal blood sugar level and for the proper course of the process of utilization of sugar by tissues. During the circulation of blood through the pancreas there occurs a very intensive absorption by it of sugar from the circulating blood, considerably exceeding the absorption by the other organs over the same interval of time.

These facts oblige one to presume that the incretion of insulin by the pancreas is accomplished without interruption, and not periodically, and that the hyperglycemia which is observed after the exclusion of the pancreas develops in the first few minutes, and not after several hours, as some researchers have hitherto considered [1, 2].

In the second part of our work we studied the role of the pancreas in the complex reflex regulation of carbohydrate metabolism in connection with the intake of food.

Experiments were conducted on an esophagostomized dog, well adapted to laboratory treatment. A cannula was applied to the pancreatic vein. In order to conduct experiments with actual and simulated feeding during partial exclusion of the pancreas from the general blood stream, the cannula was screwed on before the juncture of the pancreatic with the duodenal vein. First control experiments were conducted without the intake of food; then a study was made of the influence of simulated feeding with 500 ml of a 10% glucose solution on the course of carbohydrate metabolism during normal blood circulation in the pancreas and after its exclusion from the general blood stream. After a definite interval of time the screw of the cannula was loosened and normal blood circulation restored in the pancreas.

In these experiments the sugar and lactic acid content of the peripheral blood was studied.

The control experiments showed that the blood sugar content throughout the entire experiment (5 hours), both during normal blood circulation in the pancreas and during its partial exclusion from the general blood stream, decreased about equally. The lactic acid content of the blood decreased during the partial exclusion of pancreas from the general blood stream; after the restoration of blood circulation in the pancreas it returned to its earlier level or even somewhat exceeded it.

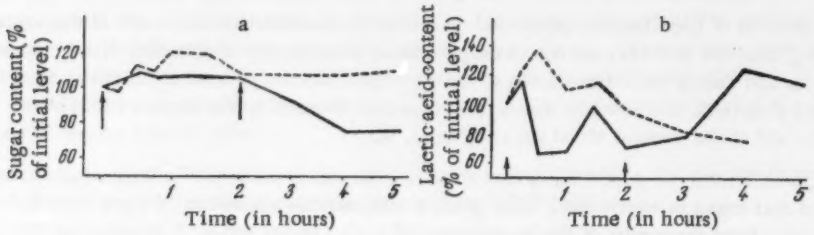


Fig. 2. The influence of simulated feeding with glucose.
a) blood sugar content; b) lactic acid content; 1) during normal blood circulation in the pancreas; 2) during partial exclusion of the pancreas from the blood circulation. The arrows indicate the beginning and end of the exclusion of the pancreas from the general blood stream.

Simulated feeding with glucose during partial exclusion of the pancreas from the general blood stream initially produced a hyperglycemic effect, as it did during normal blood circulation. After the pancreas was re-incorporated in the general blood stream, however, a significant and prolonged reduction in blood sugar content was observed (Fig. 2, a). The lactic acid content of the blood decreased after simulated feeding with the glucose solution during exclusion of the pancreas, while in the experiment without disturbance to the flow of blood from the pancreas it increased for an extended period of time (Fig. 2, b). The mechanism of this phenomenon is still not clear and further research is required.

Analysis of the data obtained after simulated feeding with the glucose solution during various conditions of blood circulation in the pancreas permits one to assume the presence of a connection between the changes in carbohydrate metabolism and the disturbance to the circulation of blood in the pancreas. Partial exclusion of

the pancreas results, after simulated feeding with glucose solution, in a reduction of the reflex phase of hyperglycemia and hypolactacidemia; the subsequent incorporation of the pancreas into the general blood stream results in hyperlactacidemia and a reduction in blood sugar content due to the excess of insulin, which during exclusion of the pancreas temporarily did not leave it to enter the circulating blood.

On the basis of our experiments the conclusion can be drawn that the effect of a rise in blood sugar after simulated feeding with glucose is accomplished with the participation of the pancreas. During partial exclusion of the pancreas from the general blood stream, the act of simulated feeding of an animal produces in it a decrease in the hyperglycemic and lactacidemic effect.

The results of the present work attest to the important role of the pancreas in the regulation of the sugar and lactic acid content of the blood and in the process of their utilization by tissues.

SUMMARY

The effect of temporary exclusion of the pancreas from the general blood circulation on the blood sugar content in the vanae pancreaticae and in peripheral blood was studied.

In critical experiments carried out on 15 cats it has been stated that the cessation of the blood flow from the pancreas brings about an increase in sugar content in the peripheral blood and its decrease in the blood of the vanae pancreaticae. In the case of free blood circulation through the pancreas, sugar content increased almost to an equal degree in both peripheral blood and vanae pancreaticae.

In series experiments on esophagostomized dogs it has been shown that, when the pancreas is excluded from the general blood circulation, simulated feeding with glucose brings about hyperglycemia and hyperlactacidemia.

These results reveal the important role of pancreas in the complex reflex regulation of carbohydrate metabolism.

LITERATURE CITED

[1] N. S. Veller and P. M. Charnaya, in the book: Abstracts of Papers Presented at the Conference Dedicated to the 100th Anniversary of the Birth of V. Ya. Danilevsky,* p. 22 (1952).

[2] N. S. Veller, P. M. Charnaya and B. S. Rodkina, in the book: Abstracts of Papers Presented at the Conference on the Problem of Cortical Regulation of the Glands of Internal Secretion,* The Academy of Sciences of the USSR, p. 21 (1953).

[3] I. S. Kanfor, in the book: Abstracts of Papers Presented at the Conference on the Problem of Cortical Regulation of the Activity of the Endocrine Glands,* The Academy of Sciences of the USSR, p. 43, (1953).

[4] I. S. Kanfor, Byull. Eksptl. Biol. i Med., No. 6, p. 7 (1955).

[5] I. S. Kanfor, Byull. Eksptl. Biol. i. Med., No. 9, p. 78 (1955).

[6] I. S. Kanfor and R. P. Olnyanskaya, in the collection: The Experimental Regulation of Physiological Functions,* The Academy of Sciences of the USSR, No. 3, p. 42.

[7] N. P. Kotshnewa, Pflug. Arch. fur d. ges. Physiol., Bd. 205, S. 482 (1924).

[8] N. P. Kotchnewa, Pflug. Arch. fur d. ges. Physiol., Bd. 201, S. 362 (1923).

[9] N. P. Kotchnewa, Pflug. Arch. fur d. ges. Physiol. Bd. 215, S. 661 (1928).

[10] E. C. London and N. P. Kotchnewa, Pflug. Arch. fur d. ges Physiol. Bd. 228, S. 533 (1931).

[11] E. C. London, Angiostomie und Organstoffwechsel, Wiem,Moskau, (1935).

[12] Grafe u. Meythaller, Schmiedeberg's Arch. exptl. Pathol. Pharmakol. Bd. 136, S. 360 (1928).

[13] E. Hedon and L. Hedon, Pancreas, secretion interne, L. Binet, Traite de physiologie normale et pathologique, Masson, t. IV, p. 19-96 (Paris, 1928).

[14] T. Kosako, J. Physiol. v. 79, N. 4, p. 416 (1933).

* In Russian.

THE SWEATING REACTIONS OF THE PALM

O. A. Krylov and E. G. Deryugina

From the Department of Human and Animal Physiology (Prof. A.N. Kabanov, chairman) of the V. P. Potemkin Moscow Pedagogical Institute

(Received April 29, 1955. Presented by V. N. Chernigovsky, Regular Member of the Academy of Medical Sciences of the USSR)

The sweating reactions of the skin of the palm differ from sweating on the remaining parts of the body in nature and intensity. V. M. Bekhterev [1] first indicated this in 1905. He observed pronounced sweating of the palm among two patients when shaking hands.

Kuno [7] and Uchino[9] place the secretion of the sudoriferous glands of the palm into a special type, emotional sweating, arising as a result of various psychic experiences (shame, fear, joy, laughter etc.) in contrast to thermic sweating on the rest of the body. In their opinion the sudoriferous glands of the palm have special sweating centers in the central nervous system which are linked with psychic activity, while the other sweating centers are linked with thermoregulation. Proceeding from the fact that the sweating reactions of the palm are extremely sensitive to psychic factors, Silverman and Powell [8] suggested evaluating various psychic illnesses according to these reactions.

The aim of our work was to ascertain the extent of the consistency of the idea that there exist distinct domains of emotional and thermoregulation sweating.

EXPERIMENTAL METHODS

Under observation were more than 300 children from one month to 12 years of age and 70 adults. Sweating intensity was determined by the electrometric method worked out by N. N. Mishchuk [3]. Sweating was measured on the palm and the back of the wrist of both hands. In a number of experiments the secretion of sweat was determined on the forehead, back, chest,and on the back and sole of the foot. More than 12,000 measurements were made in all at different times of the year. A study was made of reflex shifts in sweating under the influence of the most diverse irritants: heat effects applied to limited portions of the skin (forehead, forearm, shin, foot) by means of a special thermoirritator through which water at a definite temperature (from 0° to 60°) was conducted for $\frac{1}{2}$ -1 minute; application of heat to the entire skin surface (sun bath); changes in the surrounding temperature; physical exercises; intake of food; sleep. In separate experiments (in the summer) one hand was merely immersed in water at temperatures ranging from 18 to 24°, while sweating was studied on the other hand.

RESULTS

The sweating intensity of the palm among children up to two years is comparatively low. It increases with age, attaining maximum value from $2\frac{1}{2}$ to 7 years of age, then gradually decreases. Among adults sweating of the palm is significantly lower in the majority of cases than among children (Fig. 1).

Both among children and among adults the sudoriferous glands of the palm, as do those of the rest of the body, clearly react to temperature irritants. The peculiarity of the reactions, determined by the nature of the

irritant (stimulation of sweating with application of heat, for example, and inhibition with application of cold), precludes explaining palm sweating by the influence of only single emotions. It is sufficient to compare the sweating intensity of the palm among children 7-12 years of age during the autumn-winter period and the spring-summer period, particularly during summer on the southern shore of the Crimea, in order to have made completely clear the relation between sweat secretion of the palm and the temperature of the environment. (Fig. 2).

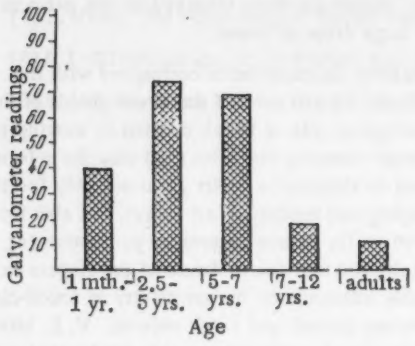


Fig. 1. Changes in the intensity of palmar sweating occurring with age (average values).

In a special series of observations we noted an increase in sweating under the influence of the emotions (reading poetry, laughter, conversation etc.) not only on the palm, but also on the back of the wrist.

Thus, according to our data, there is no basis for supposing the existence of distinct domains of emotional and thermoregulation sweating.

The sweating of the palm, however, has a number of peculiarities. The sudoriferous glands of the palm possess a higher reflex excitability; therefore the induction of sweat secretion as a result of varied stimuli (morning exercises, food intake, etc.) appears sharper and stronger, and its inhibition (for example, under the influence of cold or sleep) much weaker than on the rest of the body. It is apparent from Fig. 3 that reflex inhibition of sweat secretion as a result of brief immersion of one hand in cold water is significantly more marked on the back of the wrist than on the palm.

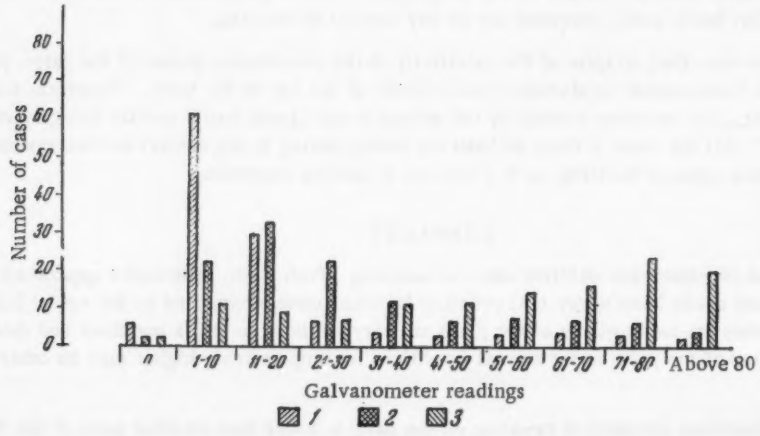


Fig. 2. Intensity of palmar sweating among children 7-12 years of age.
1) during the autumn-winter period; 2) during the spring-summer period; 3) during summer (under the conditions of the southern shore of the Crimea). The number of cases is given in percentages.

Sweating of the palm depends upon the individual peculiarities of an organism; among excitable persons it is relatively great even during sleep.

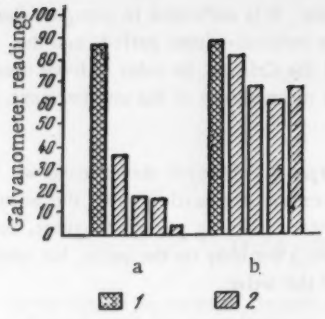


Fig. 3. Sweating on the back (a) and palm (b) of the right wrist of a ten year old child after immersion of the left hand in water at a temperature of 24°. 1) Initial level; 2) after 2, 4, 6 and 8 minutes following immersion.

Sweating of the palm in virtue of the high reflex excitability of its sudoriferous glands, is ordinarily greater than in areas of "thermoregulation sweating." In the presence of strong heat effects (the sun bath) or heavy physical exercise, the sweating of the palm, on the other hand, is lower, since the maximum output of sweat by the palm is less than that of the rest of the body. During maximum sweating the skin of the palm is covered with sweat in the form of a thin, glistening layer. Never are there observed on the palm or sole of the foot large drops of sweat.

These sweating characteristics correspond with the particular functional significance of the sweat glands of the palm, the physiological role of which consists in wetting its surface. The sweat covering the palm (and also the sole of the foot) prevents its slipping; a moist palm not only facilitates better grasping and holding of an object, but also improves the conditions for cutaneous sensory perception [4]. Again, Katz [5] showed that the moisture of the fingers exerts a considerable influence on the sensitivity of touch discrimination between smooth and rough objects. V. L. Minor [2] notes that the palmar perspiration fields are located exactly on the zones richest in receptor apparatuses.

The very form of the sudoriferous glands of the palmar surface of the fingers is adapted to lubricating the skin and softening its outer keratinized layer. Sebaceous glands are absent from the palms and soles of the feet, and the sweat, lubricating the skin, makes it more elastic, softer and less subject to injury. The ducts of the palmar sudoriferous glands form at their outlets expansions like small reservoirs or goblets in which the sweat accumulates and from which it flows out over the surface of the skin. On the other parts of the body perspiration is immediately ejected on to the surface of the skin. Such excretory dynamics promote the best evaporation of perspiration from the surface of the body. On the palms perspiration is less subject to evaporation, but is, on the other hand, easily squeezed out on any contact of the skin.

Our observations show that, in spite of the specificity of the sudoriferous glands of the palm, palmar sweating preserves the fundamental regularities characteristic of the rest of the body. Therefore, there is no foundation for separating the secretory activity of the palmar sweat glands into a special category of exclusively "emotional sweating." All the more is there no basis for distinguishing in the central nervous system special centers for each of these types of sweating, as F. Kuno and S. Uchino maintain.

SUMMARY

Studies with the electrometric determination of sweating (Prof. N. N. Mishchuk's apparatus) on a great number of children and adults have shown that sweating becomes more pronounced at the age of 2.5-5 years. It was demonstrated that the sweat glands of the plam are very sensitive to reflex reactions and therefore the intensity of perspiration of the palm under normal conditions is comparatively higher than on other parts of the body.

However, the maximal intensity of sweating on the palm is lower then on other parts of the body. That is why sweating of the palm is lower under great thermal influence.

The results obtained do not confirm the separate existence of sweating areas regulated exclusively by emotion or thermal-mechanisms.

LITERATURE CITED

[1] V. M. Bekhterev, Obozrenie Psikhiatry, Nevrology i Eksperimentalnoi Psikhology, 7, 485-486 (1905)..

[2] V. L. Minor, "Examination of Sweating by the Iodine-starch Method in Cases of Nervous Disorders and under Normal Conditions," a Thesis, Tashkent (1944).

[3] N. N. Mishchuk, "A Method of Electrometric Examination of Sweating and its Experimental and Clinical Application," a Thesis, Leningrad (1948).

[4] C. W. Darrow, Psychol. Bull. v. 33, p. 73 (1936).

[5] D. Katz, Der aufbau der Testwelt, Leipzig, (1925).

[6] T. Kosuge, Japan. J. Med. Sci. III, Biophysics, v. 5, N. 4, p. 135 (1938).

[7] F. Kuno, The Physiology of Human Perspiration, London (1934).

[8] I. I. Silverman and V. E. Powell, Am. J. Med. Sci., v. 208, N. 3, p. 297-305 (1944).

[9] S. Uchino, Japan J. Med. Sci. III, Biophysics, v. 5, N. 4, p. 134 (1938).

PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

CHANGES IN ERYTHROCYTE COUNT AND HEMOGLOBIN CONTENT IN THE PERIPHERAL BLOOD UNDER PROLONGED NOCICEPTIVE IRRITATION

V. V. Kravtsov

From the Department of Normal Physiology (Prof. S. M. Dionesov, Chairman) of the Blagoveshchensk Medical Institute

(Received August 22, 1956. Presented by V. N. Chernigovsky, Regular Member of the Academy of Sciences of the USSR)

Short-term nociceptive irritation produces in rats, rabbits and dogs an increase in the erythrocyte count and hemoblobin content in the peripheral blood [1, 7, 8, 12]. L. B. Nice and H. L. Katz [8] noted in addition that polycythemia is detected also in blood from the internal organs of rabbits, while N. S. Dzhavadyan [4, 5] made similar observations in blood from the femoral arteries of dogs.

The question of the influence of prolonged nociceptive irritation on the erythrocyte count and hemoblobin content in the peripheral blood has not attracted the attention of researchers, although the influence of just such irritation should of course, be of primary interest to the clinic.

Our task was to study the influence of prolonged (chronic) nociceptive irritation on the erythrocyte count and hemoglobin content in the peripheral blood of animals.

EXPERIMENTAL METHODS

The research was carried out on 7 dogs and 5 rabbits. For a period of 4-7 weeks determinations were made of the erythrocyte count and hemoglobin content in the peripheral blood of the intact animals. Blood was taken from a marginal vein of the ear of the animal, always from the same side, by the usual, universally adopted hematological methods. The erythrocytes in 2 drops of blood were counted, and a hemoglobin determination was made in 2 hemometers of the GS-2 type. After obtaining a steady initial erythrocyte count and hemoglobin content, the animals underwent operations for the purpose of creating a focus of prolonged (chronic) nociceptive irritation. A description of the operations is given in previous work [6].

Before and after the operation the quantity of reticulocytes in the blood was determined; changes in the reticulocyte formula according to Heilmaier's system were taken into account.

RESULTS

In a previous report [6], we showed that the number of erythrocytes among all of the dogs during the first 4-6 days after the operation increased by 500,000 per mm^3 of blood; during the first month their number increased by 2,000,000, and toward the third month was 2,500,000-3,000,000 higher than the initial level. In the subsequent months the number of erythrocytes, with certain fluctuations, exceeded the initial level by 1,500,000-2,000,000.

During the first 10-20 days after the operation the hemoglobin content increased by 28%; it remained on this level for a duration of 3 months, fluctuating sharply, but not falling to the initial value.

After 3-4 months following the operation the hemoglobin content began to decrease gradually, but remained however, 10-15% higher than the initial level; this level, occasionally with sharp fluctuations, was preserved during the subsequent months of the research.

Analagous changes were observed among the rabbits, but their onset was detected at later dates after the operations, and their expression was less distinct.

Beginning from the 25th-30th day after the operation the number of reticulocytes in the peripheral blood of the animals increased significantly: from 0.7-0.8% to 3.4-4.5% among the dogs, and from 5-8% to 16-21% among the rabbits. A shift to the left was noted in the reticulocyte formula in consequence of the appearance in the peripheral blood of reticulocytes of the 1st, 2nd and 3rd groups.

How is one to explain the significant increase in erythrocyte count and hemoglobin content in the peripheral blood under the indicated conditions ?

In the opinion of some authors [3, 10, 12], the cause of the increase in erythrocyte count during acute nociceptive irritation is the constriction of the spleen; others [1, 4, 5, 7] have shown experimentally that an increase in erythrocyte count, although a less significant one, is observed even among animals upon which splenectomies have been performed. V. N. Chernigovsky and A. Ya. Yaroshevsky [11] consider that the very removal of the spleen results among cats in an increase in erythrocyte count and hemoglobin content for a period of one month. Assuming it to be possible that the increase in erythrocyte count and hemoglobin content in our experiments occurred as a result of contraction of the spleen as the fundamental blood depot, we set out to prove the significance of the spleen in the changes in the composition of the blood during prolonged nociceptive irritation.

Two series of experiments were conducted. In the first the spleen was removed prior to the infliction of prolonged nociceptive irritation; in the other it was removed at the time of greatest change in erythrocyte count and hemoglobin content in the peripheral blood, produced by prolonged nociceptive irritation.

The results of the experiment were very much of the same type and can be illustrated by the following data.

In the dog Pika, prior to removal of the spleen, the erythrocyte count for a month amounted to from 4,800,000 to 5,200,000 per mm^3 of blood, while the hemoglobin content was 69-73 Sahli units.

After removal of the spleen we also observed, as did V. N. Chernigovsky and A. Ya. Yaroshevsky [11], an increase in erythrocyte count and hemoglobin content for 28 days after the splenectomy; after that the composition of the blood approached normal. Examination of the blood following splenectomy was continued for 2 months, after which an operation was performed which produced prolonged nociceptive irritation. On the very following day the erythrocyte count had increased by 3,300,00 per mm^3 of blood, and for a period of three months it remained higher than normal by, on the average, 1,500,000 per mm^3 with individual sharp fluctuations. From the first days of infliction of the physical irritation, the hemoglobin content increased by 30 Sahli units and remained on this level with slight fluctuations for a period of $2^1/_2$ months, after which it increased by an additional 10 units. Then after 15 days the hemoglobin content, parallel with the reduction in erythrocyte count, began gradually to decrease but remained 10-15 Sahli units above normal. Simultaneously with the increase in erythrocyte count and hemoglobin content in the peripheral blood, distinct reticulocytosis was observed: the number of reticulocytes increased from 0.65% to 4-6%. During the 6th month of postoperative examination, the number of reticulocytes fell off to 0.1%, i.e., it became less than normal (Fig. 1).

The spleen was removed from the dog Naida at the time of the significant increase in erythrocyte count and hemoglobin content produced by prolonged nociceptive irritation. The initial erythrocyte count was 5,100,000-5,500,000 per mm^3 of blood, and the initial hemoglobin content was 82 Sahli units. After the operation producing prolonged nociceptive irritation the number of erythrocytes increased by, on the average, 2,800,000, and remained on this level until removal of the spleen. On the third day following the splenectomy the erythrocyte count not only did not fall off, but even increased by 1,200,000 per mm^3 of blood; however, it quickly returned to the level prevailing before the splenectomy.

Prior to removal of the spleen the hemoglobin content was 15 Sahli units above normal, and after the splenectomy it increased by an additional 8-10 units; it remained on this level for an extended time with sharp fluctuations.

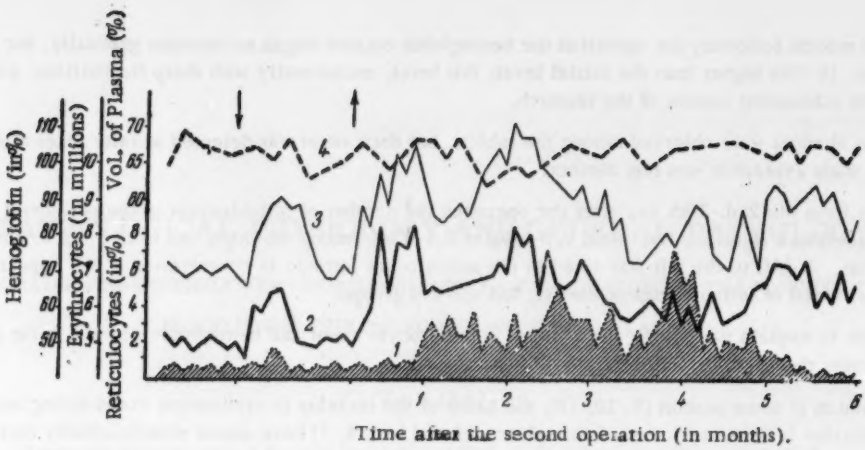


Fig. 1. The dynamics of the blood changes in the dog Pika during prolonged nociceptive irritation against a background of a removed spleen.
1) reticulocytes; 2) erythrocytes; 3) hemoglobin; 4) volume of plasma; ↓ —the operation removing the spleen; ↑ —the operation producing prolonged nociceptive irritation.

In order to check whether the increase in erythrocyte count and hemoglobin content was caused by blood clotting, we systematically examined the relative volume of the plasma with a hematocrit. It turned out that the relative volume of the plasma fluctuated within the normal limits. It can be supposed in this connection that the volume of each erythrocyte is reduced, although we have not, as yet carried out special measurements.

Fig. 2. The peripheral blood of the dog Sharik during the second month after the operation producing prolonged nociceptive irritation. In the field of vision are 1 proerythroblast, 1 basophilic and 1 polychromatophilic erythroblast and polymorphonuclear leucocytes. Ocular 8, objective 100.

Thus the removal of the spleen before or after infliction or prolonged nociceptive irritation did not eliminate the increase in both the erythrocyte count and the hemoglobin content in the peripheral blood of the animals.

How are the changes in the peripheral blood to be explained?

In order to answer this question, aside from peripheral blood smears, we examined samples of bone marrow from normal and splenectomized dogs. From the sternum and the illium 0.1-0.2 mg of bone marrow were withdrawn with Kassirsky's needle.

After 15-20 days following the operation producing the prolonged nociceptive irritation, there appeared in the peripheral blood, young forms of erythrocytes – erythroblasts with basophilic and polychromatophilic protoplasm. In one case (the dog Sharik) a proerythroblast was encountered (Fig. 2). From time to time erythroblasts were found with nuclei in a state of disintegration, a fact which attests to degenerative changes in the nuclear erythrocytes in the peripheral blood. Up to 6-7 erythroblasts on the average were encountered among 600 nucleate cells. Significant reticulocytosis was noted with a shift of the reticulocyte formula to the left; erythrocytes were encountered with Jolly bodies, in especially large amounts among splenectomized dogs.

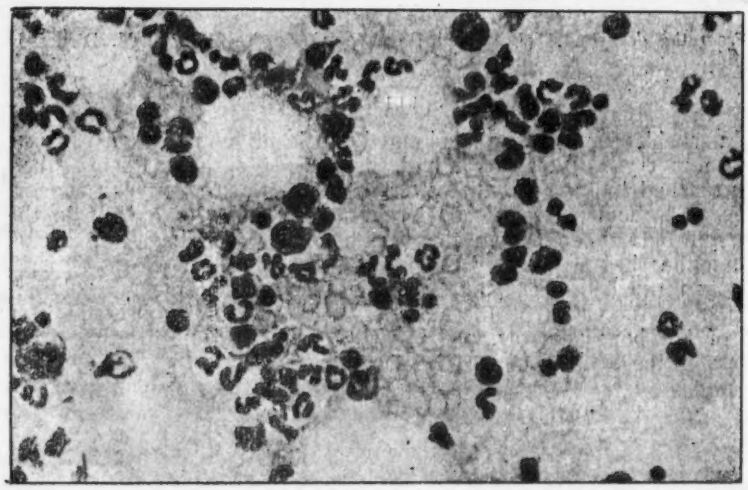


Fig. 3. Bone marrow of the dog Naida after $2\frac{1}{2}$ months following the operation producing prolonged nociceptive irritation. The proliferation of erythroblasts in the bone marrow. Ocular 8, objective 25.

In the bone marrow samples taken at different times after the operation inflicting prolonged nociceptive irritation, proliferation of erythroblasts was more or less always detected. In some cases the amount of proerythroblasts increased from 2.2 to 5.8%, the quantity of basophilic erythroblasts from 7.4 to 19.4%, and that of polychromatophilic erythroblasts from 16 to 26.6% (Fig. 3).

A relationship was also noted between the increase in the number of reticulocytes in the peripheral blood and the increase in the number of nucleate erythrocytes in the bone marrow. In this respect our data agree with the data obtained by M. I. Arinkin [2] and A. V. Solovyev [9].

The data obtained attest to the increased activity of the bone marrow, as a result of which reticulocytes of the 3rd, 2nd and 1st groups and the young forms of erythrocytes, the erythroblasts, appear in the peripheral blood of animals.

Thus it can be assumed that prolonged nociceptive irritation induces the significant generation of red

formed elements in the bone marrow, a fact which primarily explains the increase in erythrocyte count in the peripheral blood. The spleen, as the erythrocyte depot, to all appearances does not play a significant role.

SUMMARY

In rabbits and dogs prolonged nociceptive irritation causes an increase in erythrocyte count and the content of hemoglobin, the peak being reached three months after the beginning of the experiment. The number of reticulocytes is considerably increased with a shift to the left, and erythroblasts appear in the peripheral blood. The removal of the spleen either before or after the focus of prolonged nociceptive irritation has been formed, does not change the cell count. The plasma volume does not change. Increased proliferation of erythroblasts is observed in samples of the bone marrow, proving that prolonged nociceptive excitation accelerates the activity of the bone marrow.

LITERATURE CITED

[1] E. S. Andriasyan, "The Role of the Spleen in the Changes in the Morphological Composition and Coagulation of the Blood as a Result of Bleeding and Painful Irritations," a Thesis, Erivan (1945).

[2] M. I. Arinkin, The Clinic for Diseases of the Blood and Hematopoietic Organs, Leningrad (1928).

[3] I. Bulgak, "The Participation of the Spleen in the Formation of the Formed Elements of the Blood," a Thesis, Moscow (1872).

[4] N. S. Dzhavadyan, Arkh. Patol. Vol. 13, No. 1, 22-28 (1951).

[5] N. S. Dzhavadyan, Arkh. Patol. I, 22-23 (1954).

[6] V. V. Kravtsov, Trudy Blagoveshchenskogo Meditsinskogo Instituta, Vol. 2 (1956).

[7] L. B. Nice, M. E. Moris and V. P. Elhard, Cited in accordance with S. M. Dionesov, Uspekhi Sovremennoi Biol., 27, 1, 73-88 (1949).

[8] L. B. Nice and H. L. Katz, in the book: Summaries of Reports Presented at the 15th International Physiological Congress,* Moscow and Leningrad, p. 302 (1935).

[9] A. V. Solovyev, Vrachebnoe Delo, No. 1, 47-51 (1953).

[10] I. R. Tarkhanov, Trudy Sankt-Peterburgskogo Obshchestva Estestvoispytatelei, Saint Petersburg, Vol. 4, No. 1, 84-87 (1873).

[11] V. N. Chernigovsky and A. Ya. Yaroshevsky, in the book: Problems of Nervous Regulation of the Blood System,* Moscow (1953).

[12] P. A. Elgort, Trudy Astrakhanskogo Gosudarstvennogo Meditsinskogo Instituta, Astrakhan, Vol. 10, 43-46 (1955).

* In Russian.

THE INFLUENCE OF REMOVAL OF THE CEREBRAL CORTEX AND CERTAIN PHARMACOLOGICAL SUBSTANCES ON THE DEVELOPMENT AND COURSE OF FEVER•

L. I. Gorbatsevich

From the Department of General Pathology (Chairman, Prof. P. N. Veselkin, Associate Member of the Academy of Medical Sciences of the USSR) of the Institute of Experimental Medicine of the Academy of Medical Sciences of the USSR

(Received June 21, 1955. Presented by P. S. Kupalov, Regular Member of the Academy of Medical Sciences of the USSR)

The present work is devoted to a study of the role of the higher divisions of the central nervous system in the development of fever. The role of the cerebral cortex in pathological changes in thermoregulation has not yet been satisfactorily studied; exact data on the role of the cortex in fever are almost completely lacking, both with respect to its "actuating" and its "adjusting" significance. There is, however, no doubt that in an intact organism, as well as under pathological conditions, the cerebral cortex plays an important role in the regulation of all of the physiological functions of the organism.

V. M. Bekhterev [1], S. P. Botkin [2], I. L. Stychinsky [6] et al. have admitted the existence of so-called "psychogenic" fevers; a febrile increase in temperature was even obtained during hypnosis [5, 8 et al.]. It is also well-known that even mild narcosis deprives animals of their pyretic capacity [4, 7, 10 et al.]. V. Skovronsky [9] observed a sharper temperature increase among decorticated rabbits in response to intravenous injection of β-tetrahydronaphthylamine in comparison with experiments on intact animals.

In the present research a study was made of the influence of removal of the cerebral cortex on the development and course of fever (at different periods after decortication). In a number of comparative experiments a study was also made of the influence of phenocoll, bromine and sodium amytal on the development of fever among intact (normal) and decorticated dogs.

EXPERIMENTAL METHODS

Experiments were conducted on 5 dogs with cerebral cortex removed (Dezi and Lisa, about 4-5 years old; Diana, Tsygan and Mars, about 1-$1\frac{1}{2}$ years old) and on intact animals (Tuzik and Rezvy). Previously the daily fluctuations in rectal temperature of each dog had been studied; the development of the fever reaction (according to P. V. Vasilyev's method [3]) was studied before decortication (control experiments) and at different periods after unilateral and bilateral removal of the cerebral cortex.

Phenocoll was given to the dogs per os (in a dose of 0.01 g) dissolved in milk diluted by half (by 50 ml) 30-60 minutes before fever was induced; sodium bromide was administered for a week prior to the experiment and on the day of the experiment (60 minutes before its start) in doses of 0.1 and 0.5 g, also per os in milk diluted with water. Sodium amytal was injected subcutaneously in the amount of 0.05 g per kg of the animal's weight. Decortication was performed in two steps; first the cortex of the left cerebral hemisphere was removed by the usual method, and that of the right hemisphere after 2-4 months.•• The brains of the animals were

• Presented at a meeting of the Leningrad Society of Pathologists, March 31, 1953 (the research was carried out in 1951 and 1952).

•• I take the occasion to express my profound gratitude to Prof. K. S. Abuladze, an associate member of the Academy of Medical Sciences of the USSR, for supervision and assistance in performing the first operations.

subjected to a histological examination for a control of the completeness of the removal of the cerebral cortex.

RESULTS

The influence of removal of the cerebral cortex on the course of fever. The experiments of this series were conducted on 3 dogs—Dezi, Diana and Tsygan. The duration of life of the dogs after the second operation amounted to: 16 days (Dezi), 152 days (Tsygan) and 180 days (Diana). The animals finally died from general emaciation or during an attack of convulsive fits (Dezi). Following unilateral removal of the cortex, for the first month some hyperthermia was noted among the dogs, as well as greater than normal fluctuations in body temperature in the course of a day. The capacity for the pyretic state initially appeared somewhat reduced, but was quickly (after 1-2 months) restored.

Following bilateral removal of the cerebral cortex, during the first week severe motor excitation was noted among the animals, as well as a certain rise in temperature (initially), then a lowering (after 7-12 days) of temperature to the initial level. At first the animals did not take food (they were fed through tubes), did not react to the smell of meat or to sound and light irritations and moved (after 7-8 days) with a pronounced declination to the left (the animals executed a semicircle, striking objects encountered in the way). A rapid alternation was noted between a light sleep and being wide awake. On the subsequent days following the operation, an equestrian type movement was observed in walking; the animals reacted weakly to sound (a whistle or clapping of the hands) and found their food with great difficulty.

Upon dissection of Diana, for example, the following was detected: on the site of the occipital and frontal lobes—thickened connective tissue formations; in the region of the gyrus sylvioanticus and gyrus ectosylvius, the gyrus coronarius and gyrus suprasylvius—medial displacement of the internal surface of the temporal muscle; along the medial line (on both sides of the sinus sagittalis)—negligible scraps of cortical tissue delimited below from the subcortical formations by a thick connective tissue scar and above by a greatly expanded, thickened dura mater and pia mater, approaching cartilaginous tissue in consistency. Changes visible to the eye were not noted in the subcortical formations, in the mesencephalon, cerebellum and medulla oblongata. Microscopic examination (with Prof. Yu. M. Zhabotinsky's consultation) of a section from the cortical scraps (using Nissl's stain) revealed hyperemia both in all the cortical layers and in the adjacent white matter (the picture of stasis of the vessels). Altered nuclei were visible in a large portion of the nerve cells; in the pia mater a significant accumulation of hemosiderin was visible, the greater portion of which was located in macrophages, edematous modifications of nerve cells.

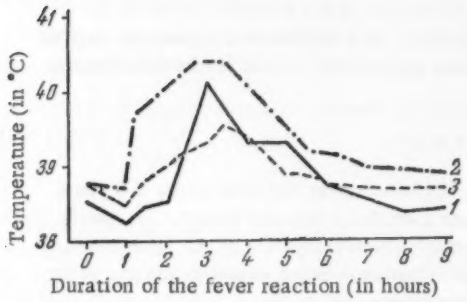


Fig. 1. The course of fever in the dog Tsygan. 1) fever reaction before the operation; 2) 10 days after bilateral removal of the cerebral cortex; 3) a month after the operation.

Following bilateral removal of the cortex, typical fever was observed 10-15 days after the operation; it was moreover, more acute (in Tsygan) in comparison with the fever in the animals subjected to unilateral removal of the cerebral cortex. When fever was experimentally induced 1, $1^1/_2$, 2, $2^1/_2$, 3 and 4 months after the operation, periodic observation revealed now the strengthening, now the weakening, of the course of the fever reaction (Fig. 1) down to temporary loss of capacity for the pyretic state. The most marked strengthening of the course of the fever was observed in experiments in which turpentine was injected into decorticated animals 2 and $2^1/_2$ months after the operation (Fig. 2).

The influence of certain pharmacological substances on the course of the fever reaction among decorticated animals. In all, 14 experiments were performed on the decorticated animals as well as 22 control experiments on intact animals: of this number, 5 experiments with phenocoll were conducted on decorticated dogs and 6 control experiments on intact dogs. The most conspicuous feature of these experiments was the difference in the behavior of intact and decorticated animals after injection of a single dose of phenocoll (10 mg): among the intact dogs there was mildly expressed

excitation and a temperature rise of 0.2-0.5°; among the decorticated animals there was clearly expressed motor excitation (a revival of equestrian movements), loud barking directly after receiving the preparation, a sharper rise in temperature (up to 2.2° in Mars, for example). A more rapid and more acute development of the fever reaction was noted in the control experiments than in the experiments without phenocoll. The duration of the fever reaction did not change noticeably. These data confirmed the results of the special experiments of Daudova, who studied the influence of phenocoll and bromine on the development of fever reaction. In the experiments on decorticated animals phenocoll also exerted a stimulating influence on the development of fever, an even clearer one in individual cases (Mars). Previous administration of sodium bromide (in a dose of 0.5 g daily for 7 days and on the day of the experiment), as in Daudova's experiments, weakened somewhat the extent of the fever reaction among the intact animals; a 0.1 g dose of sodium bromide, on the other hand, somewhat strengthened its course.

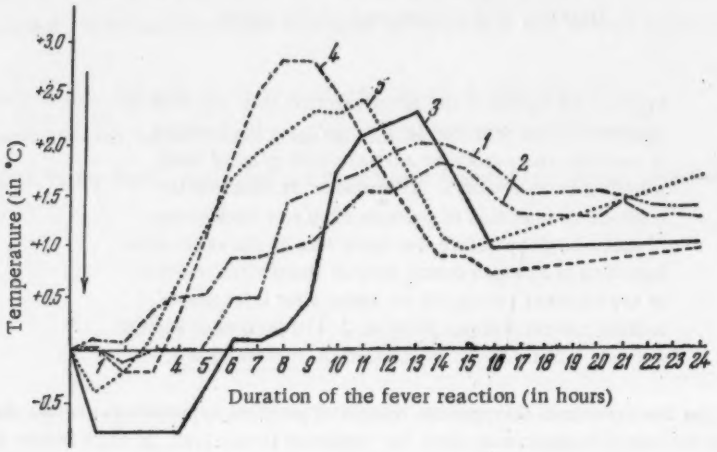


Fig. 2. The course of fever in dogs (Tsygan and Diana) when 0.5 ml of turpentine was injected subcutaneously.
1, 2, 3) fever reaction in intact dogs (Tobik, Rezvy and Lisa); 4, 5) the same in decorticated dogs 2 months (Tsygan) and $2^1/_2$ months (Diana) after the operation.

In two experiments on decorticated animals injection of sodium bromide in a dose of 0.1 g also increased the fever somewhat, while, on the other hand, there was observed a negligible decrease in fever when this substance was given in a dose of 0.5 g.

The experiments conducted with bromine are not, of course, sufficient for any conclusions to be drawn. If they receive further confirmation in the future, they could serve to point out that bromine can exert a known influence even directly on the subcortex.

Five experiments were conducted with sodium amytal on decorticated animals as well as 12 control experiments.

After injection of sodium amytal (subcutaneously in the amount of 0.05 g per kg of body weight) a lowering of temperature was noted among the intact animals (2° after 5-6 hours following injection); sleep began 1-2 hours after injection of sodium amytal and continued for 9-10 hours; injection of the same dose of sodium amytal into the decorticated dogs produced in them, at first, sudden motor excitation and a more rapid (within 15-30 minutes) onset of sleep; the sleep, however, was less deep, although more prolonged (up to 12 hours); hypothermia was also clearly evident, as among the intact dogs.

The difference in the reactions of the decorticated dogs stands out, particularly sharply in a comparison of the effect of the injection of a pyrogenic agent against a background of amytal sleep before and after the operation.

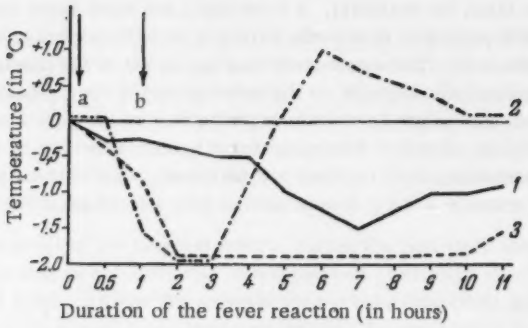


Fig. 3. The course of the fever reaction in a dog with cerebral cortex removed (Mars) against a background of narcotic sleep (sodium amytal—0.05 g/kg of body weight subcutaneously). 1) temperature reaction in response to injection of pyrogen against a background of narcotic sleep before the operation; 2) the same after injection of pyrogen during amytal sleep after removal of the cerebral cortex; 3) the same after injection of sodium amytal without pyrogen; ↓ a) injection of sodium amytal; ↓ b) injection of pyrogen (B. mesenterium).

Thus in the dog Lisa the maximum temperature rise (after previous hypothermia) before the operation amounted to 0.2° above the initial temperature; after the operation it was 1.2°. In Mars before the operation the increase in body temperature, when pyrogen was given against a background of narcotic sleep, was clear, but the temperature did not attain the initial (prior to sleep) level; in two experiments conducted after the operation, Mars' body temperature rose above the initial temperature by 0.9 and 2° (Fig. 3).

Summing up the experiments studying fever among dogs with cerebral cortex removed, one arrives at the conclusion that the fever reaction, in principle an unconditioned reflex reaction of the subcortex, is at the same time, given an entire organism, under the substantial influence of the cerebral cortex. This fact is attested to by the significant changes in the course of the fever reaction which are produced by eliminating the functional influence of the cerebral cortex on the sections underlying it.

The instability of the course of the fever reaction (now a strengthening, now a weakening) in separate periods of the lives of decorticated animals is apparently explained by processes of necrosis and cicatrization, which develop continually on the damaged surface of the brain and interfere with the activity of the subcortex, i.e., by "secondary" disturbances to the activity of the subcortex (according to I. P. Pavlov).

SUMMARY

The influence on the development and course of fever during narcotic sleep in cases of extirpation of the cortex has been studied.

Studies of the influence of phenocoll, bromide and sodium amytal on the development and course of fever in intact and decorticated dogs prove the significance of the functional condition of the higher regions of the central nervous system in the development of fever reactions and indicate a possibility of a control of its course.

LITERATURE CITED

[1] V. M. Bekhterev, "An experiment employing clinical examination of temperature in certain types of mental illness," a Thesis, Saint Petersburg (1881).

[2] S. P. Botkin, Clinical Lectures, Saint Petersburg, No. 1 (1885).

[3] P. V. Vasilyev, Byull. Eksptl. Biol. i Med., Vol. 27, No. 2, 139-140 (1949).

[4] N. N. Misyul, The Mechanisms of Pathological Reactions* (Leningrad 1949) issue 11-15, pp. 21-38 (1949).

[5] K. I. Platonov, in the book: Psychotherapy,* Kharkov, 11-122 (1930).

[6] I. L. Stychinsky, Klin. Med. issue 5-6, pp. 44-46 (1923).

[7] G. Tarasevich, "The problem of the treatment of septicemia with chloral hydrate," a Thesis, Saint Petersburg (1880).

[8] Eichelberg, Dtsch. Zeitschr. f. Nervenheilk, Bd. 68-69, S. 352-356 (1921).

[9] V. Skowronski, Arch. exper. Pathol. Pharmakologie, Bd. 146, S. 1-19 (1929).

[10] R. Thauer, in the book: Ergebnisse der Physiologie Biologischenchemie, Munchen, Bd. 41, S. 607-805 (1939).

* In Russian.

THE SEQUENCE OF THE DISAPPEARANCE OF OCULOMOTOR REFLEXES IN TRAUMATIC SHOCK*

V. D. Lindenbraten (Leningrad)

From the S. M. Kirov Military Medical Academy

(Received January 22, 1955. Presented by S. N. Davidenkov, Regular Member of the Academy of Medical Sciences of the USSR)

We studied the general state and functional disturbances of the activity of the brain stem of animals during and immediately after prolonged pinching of the soft tissues of the limbs (the thigh muscles of rabbits were pinched for four hours in a vise). During the course of the experiments a record was kept of blood pressure, respiration, temperature, vasculomotor and oculomotor reflexes. In all, 44 experiments were conducted.

In the present report the question of the sequence of the disappearance of occulomotor reflexes is considered, a question which bears a direct relationship to the problem of the unequal resistance of different brain structures.

Oculomotor reflexes were produced by moving the bodies of the animals to the right and left of the median line while the head was held immobile. The eye muscles (external and internal rectus, the superior oblique and in some experiments even the muscle of the nictitating membrane) were connected with small rods which recorded the contractions of the muscles on a smoked kymographic sheet. The method of recording the oculomotor reflexes has been described in detail by P. A. Gultyaev [2].

In all of the experiments the extraordinary stability of the oculomotor reflexes was apparent. This fact was noted also in the work of N. G. Savvin [15]. Studying the influence of hypoxemia on the functional state of antagonistic eye muscles, he discovered that in many cases nystagmus persists up to the cessation of respiration. In a number of our experiments the amplitude of the oculomotor reflexes several minutes before the demise of the animals was not lower than the initial level (Fig. 1).

In virtue of the fact that the nuclei of the oculomotor nerves, which innervate the internal rectus muscles, and the nuclei of the trochlear nerves, which innervate the superior oblique eye muscles, are located in the mesencephalon, while the nuclei of the abducens nerves, which innervate the external rectus muscles, are located in the medulla oblongata, it was possible for us to control to a certain extent the functional state of these two divisions of the brain.

The notion of the many-storied construction of the nervous system and of centers of nervous activity corresponds to the study of I. P. Pavlov and to the work of V. M. Bekherev and A. A. Ukhtomsky. Observing the fading away of the functions of the central nervous system in the deep torpid phase of shock, we assumed that the reflexes of the internal rectus muscle disappeared earlier than the reflexes of its antagonist. In none of the experiments conducted, however, were we able to note such a sequence. On the contrary, the reflexes of the internal rectus muscle in a number of experiments disappeared last, directly before the cessation of respiration (Fig. 2).

* Presented at the Scientific Conference of Physicians of the Central Far-Eastern Military Hospital, April 18, 1954.

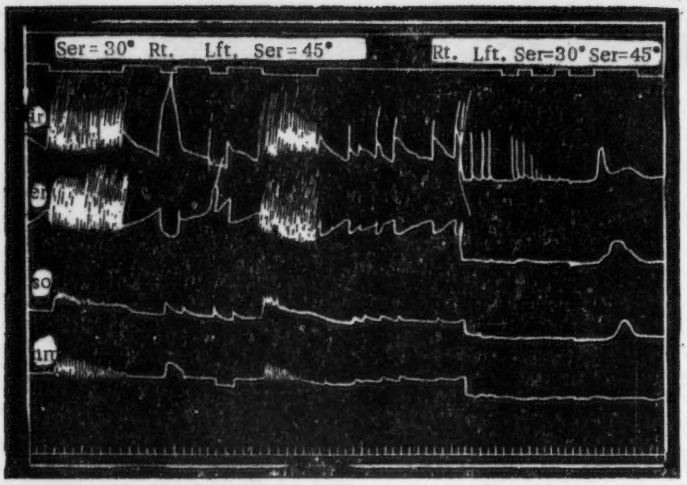


Fig. 1. Kymogram of oculomotor reflexes. Reflex stability. Five minutes before the death of the animal the amplitude of its reflexes is higher than the initial level. The independent contractions of the internal rectus muscle of the eye before the death of an animal are visible. The reflexes of the superior oblique and external rectus muscle as well as the muscle of the nictitating membrane, are absent at this time.
Significance of the curves (from the top down): the record of single (rt, lft) movements and serial (Ser. = 30°, Ser. = 45°) movements of the body of an animal to the right and left of the median line; ir) contraction of the internal rectus muscle; er) contraction of the external rectus muscle; so) contraction of the superior oblique muscle; nm) contraction of the nictitating membrane; unit of the time scale) 5 second.

The transformation of the rhythm of the irritation into a more abrupt rhythm of contractions (Fig. 3) and the inhibitory phase of the reflexes (Fig. 4) were most often apparent in the superior oblique and external rectus muscles. Out of 8 experiments in which we attempted to apply a complex method of therapy in order to free the animals from the state of shock, the stepping up of the reflexes of the superior oblique muscle was noted in only one case, while the reflexes of the external rectus muscle were stepped up in 6 cases, and the reflexes of the internal rectus in 7 cases.

In addition, the reflexes of the superior oblique muscle almost always died out earlier than the other oculomotor reflexes. The nuclei of the trochlear nerves, located in the mesencephalon, were the first to be shut down, then the nuclei of the abducens nerves, located in the medulla oblongata, and the very last were the nuclei of the oculomotor nerves, located in the mesencephalon, i.e., certain nuclei of the mesencephalon. Upon further analysis of the experimental material, taking into account all the facts obtained, it turned out that not only the sequence of disappearance of the oculomotor reflexes, but also the dynamics of their changes in the course of the development of shock, attest to the considerable resistance of the nuclei of the oculomotor nerves in comparison with the nuclei of the abducens and trochlear nerves. Thus, the fluctuations in the magnitude of the reflexes of the internal rectus muscle in the basic series of experiments devoted to a study of the changes in oculomotor reflexes were significantly less than the fluctuations of an analagous character of the external rectus and, especially, the superior oblique muscle of the eye. The average value of the step-up of reflexes, which was as a rule, observed in the initial period of pinching, was also appreciably less for the internal rectus muscle. This is reflected in the Table.

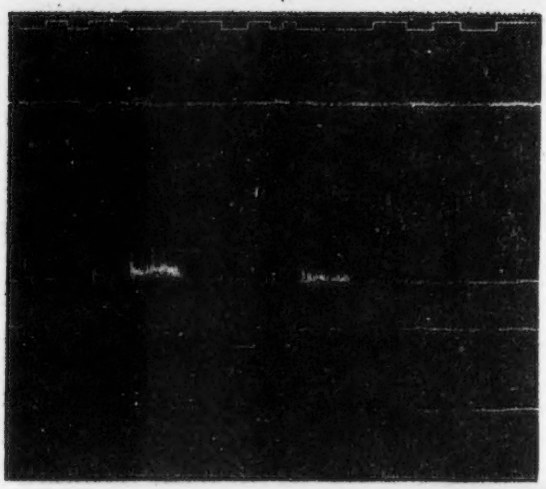


Fig. 2. Kymogram of oculomotor reflexes. The sequence of the shutdown of reflexes. The significance of the Curves is the same as in Fig. 1.

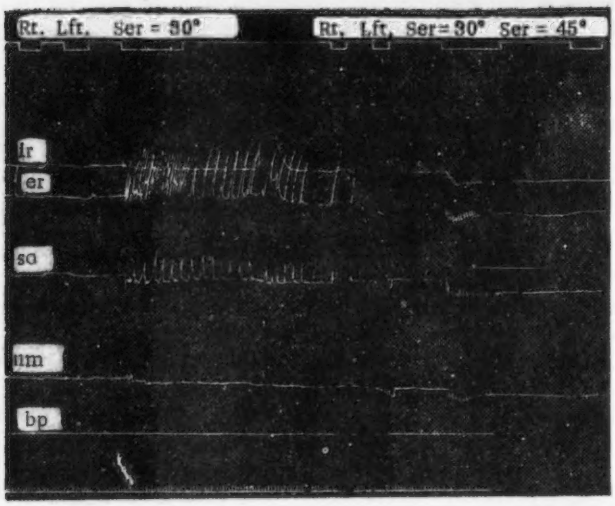


Fig. 3. Kymogram of oculomotor reflexes. The transformation of rhythm. The significance of the curves is the same as in Fig. 1. bp) blood pressure record (before the death of the animal the blood clotted in the cannula).

The question of the sequence of disappearance and restoration of the activity of the different divisions of the brain in grave traumas, loss of blood and other pathological processes has attracted the attention of researchers in connection with the study of the problem of the revival of an organism. Some authors, having observed the restoration of the functions of the medulla oblongata following a 10-15 minute disruption of blood circulation in the brain, maintained that after the indicated time, full restoration of the functions of the entire central nervous system was possible.

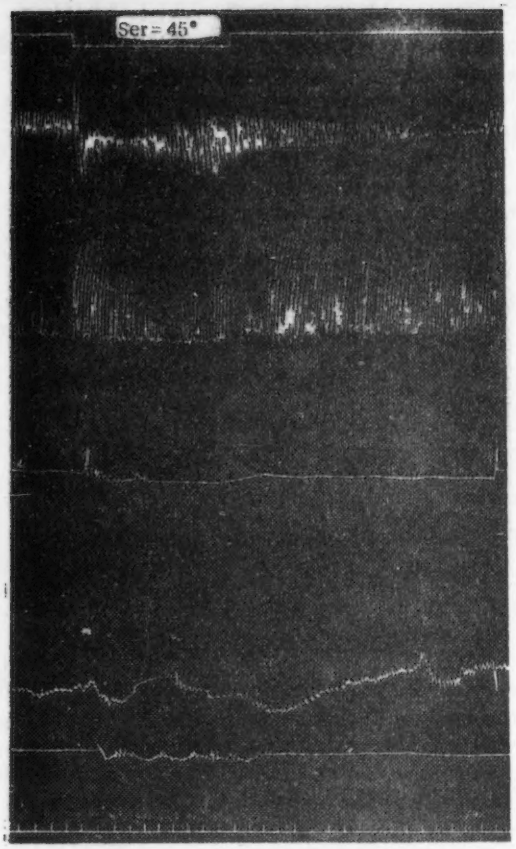


Fig. 4. Kymogram of oculomotor reflexes. The inhibitory phase. The significance of the curves is the same as in Fig. 1.

The fallacy of this sort of notion was shown by the work of I. R. Petrov [14], V. A. Negovsky [7], Heymans and Boukaert [17] and others. In experiments on animals, as well as by clinical observations of humans, it was established that the order of the exclusion or restoration of this or that division of the brain is determined by its phylogenetic age: the younger structures are eliminated earlier and are reinstated later. There appeared the idea of the fading and restoration of the divisions of the brain "by floors."

However, even in the early works of I. R. Petrov [12, 13] he mentions exceptions to this general rule. It turned out that certain functions intrinsic to the higher divisions are restored earlier than those of lower divisions of the brain. The author connects this with the nidal quality of afflictions of the central nervous system. In addition to this, it was discovered that the revival of the formations of the same level also does not occur simultaneously. In the research of V. A. Negovsky [7, 8, 9, 10] it is noted that, long before full restoration of the function of the entire diencephalon, signs are observed of the restoration of the functions of the extrapyramidal system.

The amazing stability of the ocular reflexes is attracting attention. According to the data of A. A. Narychev [6], who studied the pupillary reflexes of humans under narcosis during operations, the reaction of the pupil to light disappears only after paralysis of the respiratory center. In the experiments of E. I. Garber [1] on frogs, in the process of dying, the corneal reflex disappeared also only after breathing stopped.

Comparing our data with the observations of other authors, we came to the conclusion that the hypotheses advanced to explain the deviations from the rule of revival "by floors" of the different divisions of the brain do not enable us to explain the results of our experiments conducted. The premature falling off of certain reflexes could be linked with the nidal quality of affliction, but their increased resistance could not, since in the latter case one would have to admit the presence of continuous "nidi" of infection around an undamaged area. At the same time, the later disappearance of a number of reflexes (oculomotor reflexes of the internal rectus muscle of the eye, reflexes of the pupil to light) which are affected via the mesencephalon, in comparison with reflexes effected via the medulla oblongata, is evidence of increased resistance of certain centers of the mesencephalon. The reference of V. A. Negovsky to the greater stability of the parasympathetic nervous system in contrast to the cerebrospinal system has no relation to the facts adduced, inasmuch as only somatic motor reflexes were recorded by us. There are indications [17] that the innervation of the external rectus muscle of the eye appeared earlier in the process of evolution than the innervation of the internal rectus muscle. Thus, even the age criterion does not clear up the situation.

What is the reason for the increased resistance of a number of reflexes connected with the mesencephalon?

The greater stability of certain reflexes effected with the participation of higher centers, in comparison with reflexes connected with lower centers, is apparently explained by the greater biological significance of the

former. This resistance was consolidated in the course of evolution since, to a considerable extent, it guaranteed the survival of an animal in various difficult situations, and thereby favored preservation of the species. Thus, between the resistance of a system and its biological significance there exists a direct connection, which determines the sequence of the shutdown of the different divisions of the brain during the expiration of an organism and of their restoration during its revival. The rule of a shutdown "by floors" is an objective expression of the fact that the lower divisions of the brain have a greater biological significance for a dying organism than the higher divisions.

The Change in Reflexes of the Eye Muscles After Pinching of a Limb

Muscle	Limits of the fluctuations in the magnitude of the reflexes (in % as compared with the initial value)	Mean value of the step-up of reflexes (in % as compared with the initial value)	The number of experiments in which the development of an inhibitory phase was observed.
Internal Rectus	138	21	4
External Rectus	180	36	6
Superior Oblique	215	56	8

* Note: The numbers express the mean values of the fluctuations. Total number of experiments, 23.

According to the data of E. K. Senn [16], the development of the mesencephalon is closely connected with the development of the visual analyzer, that most important sense organ. Particular significance in guaranteeing the full-scale work of the visual organ was acquired by the system of oculomotor nerves, which has a complex structure and which guarantees the accomplishment of a number of important visceral and somatic functions. The physiological significance of the functional performance of this system apparently also led to securing its increased resistance in the course of evolution. The reflexes which are effected with the participation of the trochlear nerves and the nerves of the nictitating membrane (the motor nuclei of which, according to the data of G. L. Komendantov [4], are also located in the mesencephalon) are less important with respect to physiological role than the reflexes realized with the participation of the oculomotor nerves. This clearly also explains the fact that the times of the shutdown of the indicated reflexes keep within the rules of a shutdown "by floors" of the divisions of the brain.

SUMMARY

In a study of the deep torpid shock phase on rabbits it was brought out that oculomotor reflexes of the internal and external rectus muscles, superior oblique muscle and muscle of the nictitating membrane are characterized by a high stability: they disappear just before the cessation of respiration, maintaining significant amplitude till their very disappearance. Reflexes of the superior oblique muscle, realized through the mesencephalon are the first to disappear, they are followed by those of the external rectus realized through the medulla oblongata; the reflexes of internal rectus, realized through the mesencepalon are the last to disappear.

Not only visceral, but also somatic centers of the mesencephalon, particularly the centers of oculomotor nerves, possess higher stability in comparison with certain centers of the medulla. This characteristic is apparently connected with their great physiological importance.

LITERATURE CITED

[1] E. I. Garber, Arkh Patol., No. 2, 59-69 (1949).

[2] P. A. Gultyaev, "The comparative characteristics of the influence of ether, chloral hydrate, hexobarbital, urethane and morphine on the reflex activity of an animal," a Thesis, Leningrad (1952).

[3] I. V. Davidovsky, Problems of Localization and Organopathology in Light of the Teaching of Sechenov, Pavlov and Vvedensky,* Moscow (1954).

[4] G. L. Komendantov, Fiziol. Zhur. SSSR, Vol. XXXIV, No. 4, 449-456 (1948).

[5] V. P. Kurkovsky and I. R. Petrov, in the book: Oxygen Hunger and the Struggle Against It,* Leningrad, 3-48 (1940).

[6] A. A. Narychev, Arkh. Patol., Vol. XV, No. 6, 77-81 (1953).

[7] V. A. Negovsky, The Restoration of the Vital Functions of an Organism in the State of Agony or during Clinical Death,* Moscow (1943).

[8] V. A. Negovsky, Experimental Therapy on the States of Agony and Clinical Death in a Military District,* Moscow (1945).

[9] V. A. Negovsky, Arkh. Patol., Vol. XV, No. 4, 3-14.

[10] V. A. Negovsky, The Pathophysiology and Therapy of Agony and Clinical Death,* Moscow (1954).

[11] I. R. Petrov, Fiziol. Zhur. SSSR, Vol. XXIV, No. 1, 34-48 (1936).

[12] I. R. Petrov, Arkhiv Patologicheskoi Anatomy i Patologicheskoi Fiziology, Vol. III, No. 3, 12-36 (1937).

[13] I. R. Petrov, in the book: A Collection of Memory Difficulties from the I. P. Pavlov Academy,* 63-73, Leningrad (1938).

[14] I. R. Pavlov, Klin. Med., Vol. XVII, No. 11, 6-24 (1939).

[15] N. G. Savvin, in the book: Transactions of the Military Medical Academy,* Moscow, Vol. 1, 214-224 (1946).

[16] E. K. Sepp, A History of the Development of the Nervous System of Vertebrates from the Acraniates to the Human,* Moscow (1949).

[17] C. Heymans and J. J. Boukaert, Fiziol. Zhur. SSSR, Vol. XXI, No. 5-6, p. 770 (1936).

* In Russian.

BIOCHEMISTRY AND BIOPHYSICS

THE HALF-LIFE OF THE DIFFERENT SERUM PROTEIN FRACTIONS IN THE CIRCULATING BLOOD

M. S. Surovikina

From the Department of Pathological Physiology (Prof. I. A. Oivin, Chairman) of the Stalinabad Medical Institute and the Department of Biology and Chemistry (Lecturer L. Sh. Radzhabov, Chairman) of the Stalinabad Pedagogical Institute

(Received May 4, 1956. Presented by Prof. A. E. Braunshtein, Regular Member of the Academy of Medical Sciences of the USSR)

The possibility of artificially labeling protein preparations, the natural serum proteins in particular, permits a study of the duration of life of the different protein fractions and thus, to judge the intensity of the processes of their synthesis and destruction.

Certain researchers, using protein preparations labeled with I^{131}, determined the half-life of albumin and γ-globulin in humans and in a number of animals [6, 7, 8]. On the basis of these data it was computed that in the rabbit, for example, 0.6 g of serum albumin and globulins is synthesized and destroyed daily [9].

The utilization of different protein preparations, however, does not permit one to answer the question of the duration of life of all the protein fractions of blood serum; furthermore, preparative separation of the protein fractions may tell on the properties of the proteins. Natural homologous serum proteins were therefore employed.

The aim of our work was to determine the half-lives of the different serum protein fractions, labeled with I^{131} and S^{35}, in the circulating blood of rabbits.

Intravenously injected serum proteins are cleared from the blood stream according to a double exponential curve [10, 13, 15, 16]. The first part of the curve characterizes the process of establishing a dynamic equilibrium between the proteins of the blood and the proteins of the extracellular fluid, while the second phase is dependent upon the processes of metabolism and the destruction of the serum proteins. According to our data, the time of half-clearance of the protein fractions labeled with both I^{131} and S^{35} from the vascular system of the rabbit in the first distributive phase equals, on the average, $3\frac{1}{2}$-5 hours (the first exponential portion of the clearance curve). After 24 hours the reduction in the protein content of the blood is retarded and depends upon processes of their destruction. We therefore carried out the determination of the duration of the half-life of the different serum protein fractions of the blood in the second stage of their clearance from the blood stream (the second exponential portion of the curve), which begins a day after intravenous injection of the labeled serum proteins.

EXPERIMENTAL METHODS

Two series of experiments were conducted: the first on 5, the second on 4 rabbits of different color and sex weighing $1.77\sigma \pm 0.22$ kg. In the first series of experiments, serum from the blood of donor rabbits (8-15 ml) labeled with I^{131} outside of the organisms was injected into the experimental rabbits; in the second series the serum of donor rabbits (15-20 ml) labeled within the organisms with S^{35} was used. Following a single injection of the labeled serum, blood was taken from the experimental rabbits every 24 hours for a period of 5 days.

The serum specimens were studied by the method of paper electrophoresis. Applied to a strip of paper was 23.1 ± 0.1 mg of serum. The protein separation took place over a period of $2\frac{1}{2}$ hours using 19 v/cm and 1 mA/4 cm. A barbital buffer was used (pH = 8.6, μ = 0.023). The apparatus is described in the work of E. P. Smolichev [2]. The paper was stained with a solution of bromophenol blue. The stained electrophoregram was cut into smaller strips 0.5 cm wide. Each of these strips was again cut into 3 parts and placed in a standard target (15 mm) under a Type B end counter apparatus, and the radioactivity was computed for a period of 5-10 minutes. According to the results of the computation the average background values were calculated. Then the concentration of the dye eluted from each strip with 0.01 NaOH was determined in a multistage photometer. Electrophoretic curves were constructed on the basis of the results expressed in units of absorbance. The total radioactivity of the separate protein fractions was obtained by adding the activities of the individual strips which went to make up a fraction. In computing the radioactivity of the protein fractions a correction was made for the value of the radioactive trace [2]. The activity of the whole serum (23.1 mg), which was applied to a standard target, was computed simultaneously.

The experimental results were expressed in the period of half-life of the different labeled protein fractions in the blood stream. Half-life was determined by means of a graph constructed with the semilogarithmic system of coordinates [1].

Iodination of the serum proteins was performed according to the method of Francis, Mulligan and Wormall [11]. In some of the experiments the serum was freed from the uncombined I^{131} by dialysis into an 0.9% NaCl solution, in others by passing the serum through the anionite EDE-10. The radioactivity of the serum after dialysis amounted to 200,000-250,000 counts/min./ml.

In order to obtain S^{35}-labeled serum the donor rabbits were injected with fractional doses (4-5 times) of 1.5-2 mc of methionine containing S^{35}. Twenty-four hours after the first injection of the isotope the rabbits were exsanguinated via the carotid artery and the serum was obtained. About 5% free methionine was contained in the serum. The radioactivity of the S^{35}-labeled serum amounted to 55,000-85,000 counts/min./ml.

The labeled serum specimens were kept in a refrigerator not more than a day and were heated to 36° prior to intravenous injection.

RESULT OF THE EXPERIMENTS

The results of the first series of experiments are listed in Table 1.

TABLE 1

Half-life in the Blood Stream of the Different Serum Protein Fractions Labeled with I^{131} (in days)

No. of the Experiment	Protein fractions					Proteins of whole serum
	Albumin	Globulins				
		α_1	α_2	β	γ	
1	6.2	2.2	2.0	2.5	2.2	4.9
2	3.0	2.8	3.3	2.9	3.5	4.5
3	4.5	3.8	4.4	6.0	4.1	4.5
4	5.3	3.0	3.5	2.2	3.6	3.7
5	5.2	4.1	4.3	4.4	5.0	4.0
M	4.8	3.2	3.5	3.6	3.7	4.3
$\sigma\pm$	1.19	0.77	0.96	1.59	1.02	0.47
M±	0.53	0.34	0.043	0.71	0.46	0.21

A statistical analysis of Table 1 shows that the half-life in the blood stream of all of the globulin fractions (α_2-, β and γ-globulins) is the same ($P > 0.05$) and equals, on the average, $3\frac{1}{2}$ days. The half-life of albumin is somewhat longer ($P < 0.05$), averaging $4\frac{1}{2}$ days. The mean half-life of the proteins of whole serum amounts to 4-$4\frac{1}{2}$ days.

The results of the determinations in the second series of experiments are shown in Table 2.

TABLE 2

Half-life in the Blood Stream of the Different Serum Protein Fractions Labeled with S^{35} (in days)

No. of the Experiment	Protein fractions					Proteins of whole serum
	Albumin	Globulins				
		α_1	α_2	β	γ	
1	3.9	5.0	2.7	2.8	3.7	4.0
2	4.5	3.4	4.0	4.2	3.8	4.3
3	3.6	4.7	4.4	3.0	3.6	6.0
4	6.0	3.3	3.0	3.8	4.0	5.2
M	4.5	4.1	4.0	3.4	3.8	5.1
$\sigma\pm$	1.07	0.88	0.98	0.66	0.18	0.87
$M\pm$	0.53	0.44	0.48	0.33	0.09	0.44

We admitted the statistical handling of such a small number of experiments in view of the fact that each of the values presented in Table 2 is the average of 5 measurements.

A comparison of the data included in Tables 1 and 2 showed that the half-life of the protein fractions labeled within an organism with S^{35} and outside of an organism with I^{131} is the same and amounts to $4^1/_2$ days for albumin and $3^1/_2$-4 days for the globulins. If it is assumed that the volume of a rabbit's blood adds up to $^1/_{13}$th of its weight, that the hematocrit reading amounts to 41.5% [5], the concentration of protein in the serum to 6.3% g (60.5% albumin and 39.5% globulins [4]), the half-life of albumin to $4^1/_2$ days, that of the globulins to 4 days and that of the proteins of whole serum to $4^1/_2$ days, then about 0.3 g of serum proteins (0.18 g of albumin and 0.12 g of the globulins) per kg of body weight must be synthesized and destroyed daily in the rabbit organism. This agrees with the data of Dixon and Maurer [9].

SUMMARY

Determination of the half-life of the single serum protein fractions in the circulating blood of the rabbit has been carried out by means of electrophoresis on paper combined with radiometry. The half-life of natural serum proteins labeled outside the organism with I^{131} and within the organism with S^{35} has been studied. Both experimental series revealed that the average half-life of all the globulin fractions attained 3.5 to 4.0 days, those of albumin —4.5 days. 0.3 g of serum proteins (0.18 g of albumin and 0.12 g of globulin) per kg of weight are, therefore, daily synthesized and destroyed in the rabbit organism.

LITERATURE CITED

[1] I. A. Oivin, in the book: Transactions of the Stalinabad State Medical Institute, Stalinabad, Vol. 21, pp 195-230 (1956).

[2] E. P. Smolichev, ibid., pp. 237-253.

[3] M. S. Surovikina, ibid., pp. 33-37.

[4] M. S. Surovikina, in the book: Transactions of the Stalinabad Medical Institute, Stalinabad, Vol. 13, pp 85-88 (1954).

[5] E. C. Albritton, Standard Values in Blood, Philadelphia and London, p. 42 (1953).

[6] A. Bauman, M. A. Rothschild, R. S. Yalow and S. A. Berson, J. Clin. Invest., V. 34, pp 1359-1368 (1955).

[7] F. J. Dixon, P. H. Maurer and M. P. Deichmiller, Proc. Soc. Exp. Bio. and Med., V. 83, N. 21, pp 287-288, (1953).

[8] F. J. Dixon, D. W. Talmagl, P. H. Maurer and M. J. Deichmiller, J. Expti. Med., V. 96, No. 4, pp. 313-318 (1952).

[9] F. J. Dixon and P. H. Maurer, Expti. Med., V. 101, No. 3, pp. 233-244 (1955).

[10] L. L. Forker, I. L. Chaikoff and W. O. Reinhardt, J. Biol. Chem., V. 197, pp 625-636 (1952).

[11] G. Francis, N. Mulligan and A. Wormall, Nature, V. 167, pp 748-751 (1951).

[12] P. H. Maurer and A. Niklas, in the book: Radioaktive Isotope in Klinik und Forschung, Munchen – Berlin, S. 202-211 (1955).

[13] L. R. Melcher and S. P. Masouredis, J. Immunol. V. 67, pp 393-402 (1951).

[14] A. Niklas, W. Maurer and H. Krause, Biochem. Ztschr. Bd. 325, S. 464-476 (1954).

[15] K. Wasserman and H. S. Mayerson, Amer. J. Physiol. V. 165, pp 15-26 (1951).

* In Russian.

THE METABOLISM OF HYDROGEN SULFIDE (H_2S^{35}) INJECTED SUBCUTANEOUSLY

A. I. Gunina

From the Biochemical Laboratory (Head – Senior Research Worker A. I. Gunina, Scientific Director – Prof. I. A. Oivin) of the Sochy Rheumatism Institute (Director – Lecturer N. P. Vladimirov)

(Received June 26, 1956. Presented by S. V. Anichkov, Regular Member of the Academy of Medical Sciences of the USSR)

The metabolism of exogenous hydrogen sulfide in an organism has been studied by a number of authors [3, 4, 5, 6, 7, 9, 10]. It has been established that hydrogen sulfide is rapidly oxidized and is transformed on the whole into pharmacologically inactive sulfates. Insufficient light has been shed on the question of the possible inclusion of exogenous hydrogen sulfide or its oxidation products in the proteins of organs and tissues. Tarver and Schmidt [10] noted that S^{35}-sulfates do not enter into the construction of the sulfur-containing amino acids. Smith, Anderson and Odell [9] have shown that intravenously injected S^{35}-sulfates are combined in the blood with a compound possessing electrophoretic mobility similar to the mobility of α_1-globulin. The authors consider that this compound is a protein with a sulfur-containing carbohydrate prosthetic group.

The aim of our work was to study the metabolism of hydrogen sulfide containing S^{35} after subcutaneous injection of Na_2S^{35}.

The direct injection of large amounts of sulfide into the blood is limited by its high toxicity. Significantly greater amounts of sulfide can be injected subcutaneously than can be injected intravenously. According to V. A. Tikhonravov and A. N. Iskova [7] lethal doses of hydrogen sulfide in the case of subcutaneous injection are 10-35 times larger than in the case of intravenous injection. This is explained by the fact that the hydrogen sulfide, gradually permeating from the subcutaneous tissue into the blood, has time to be oxidized and does not reach toxic concentrations in the blood.

EXPERIMENTAL METHODS

Experiments were conducted on 34 male white rats weighing from 160 to 230 g. Na_2S^{35} was injected subcutaneously in the abdominal region in a single dose corresponding to 0.2-0.5 mg of H_2S^{35} (from 6×10^5 to 1.7×10^6 impulses/min). In some of the experiments Na_2S^{35} was injected daily for a period of 4-5 days (in all from 3×10^5 to 11×10^6 impulses/min were injected during an experiment). Rats were decapitated 1, 2, and 6 days after injection of Na_2S^{35}. A determination was made of the H_2S^{35} in the blood, organs and urine. The distribution of S^{35} was studied in dry preparations of the organs, blood and urine as well as in the proteins of the organs. In order to calculate specific activity the total sulfur content of the organs was determined. The overall amount of sulfur and inorganic sulfates in the urine was determined.

For the H_2S determination blood or ground tissue was acidified, and the H_2S forced out with a stream of nitrogen into two receiving vessels containing 2 ml of 0.1 N NaOH. The determination of H_2S in the alkaline solution was performed according to the Karo-Fischer method. The details of the determinations were described earlier by us and by V. A. Tikhonravov [1]. Aside from the photometric determination of H_2S an impulse count

was made of the receptacle. The sensitivity of the chemical method under the conditions of the technique employed amounted to 0.04 γ H_2S per ml, while that of the radiometric method was 0.002-0.0004 γ H_2S^{35} per ml.

The determinations of the urine sulfur fractions were performed by the gravimetric method. The total sulfur content of the organs was determined by the gravimetric method with prior hydrolysis by boiling for 10-12 hours in 10% NaOH. Barium sulfate was collected by centrifuging in special conical test tubes. The samples for the count were prepared in a kind of suspension (2.5 mg $BaSO_4$ and 0.05 ml of 70% alcohol), which was distributed uniformly over a target. The preparations were dried slightly at 50-60°.

Natural preparations were prepared for a count from organs finely sliced and ground in a mortar (0.1-0.3 g). The count was made after slight drying. The protein preparations were prepared by the ordinary method of precipitation with trichloroacetic acid. They were washed in a liquid rinse prior to the disappearance of radioactivity. The dried preparations were triturated, sifted and placed in 50 mg amounts in tin foil targets (of the size of a 10 kopeck coin). The count of the activity of all the specimens was carried out in a lead booth by means of an end counter. The duration of the count was determined by the magnitude of the activity of the specimen (from 5 to 15 minutes). The radioactivity counts were made several times during the working day. The sodium sulfide containing radiosulfur included an admixture of sulfates. Therefore, in order to standardize it and purify it of contaminants, before each experiment the solution was acidified and H_2S was forced out into 0.1 N NaOH. Such freshly prepared Na_2S^{35} solutions were used in all of the experiments.

RESULTS OF THE EXPERIMENTS

The hydrogen sulfide content of the blood was determined in 22 rats. H_2S was not detected in a single case, neither by the chemical nor the radiometric method. Analogous results were obtained in the examinations of the lungs and urine. Hydrogen sulfide was found in the liver in 5 cases by the chemical method, but was not detected in a single case by the radiometric method.

The hydrogen sulfide detected in the liver we consider to be endogenous, since hydrogen sulfide was not infrequently found in the liver of normal rats without its injection from without. Had the subcutaneously injected hydrogen sulfide been present in the liver, it would have been detected by the radiometric method.

TABLE 1

Elimination of S^{35} among Rats after Subcutaneous Injection of NA_2S^{35}

Time after injection in days	Experiment 1				Experiment 2			
	Na_2S^{35} was injected subcutaneously							
	220 γ (744 × 10^3 impulses/min)				182 γ (602 × 10^3 impulses/min)			
	Total sulfur, imp/min/ml	Radioactivity in %		% of the injected number of imp./min	Total sulfur, imp/min/ml	Radioactivity in %		% of the injected number of imp/min
		inorganic sulfates	neutral sulfur			inorganic sulfates	neutral sulfur	
1	71 786	97.8	2.2	55	—	—	—	—
2	10 681	92.3	7.7	5.5	2916	87.1	12.9	3.1
3	—	—	—	—	336	65.2	34.8	0.84
4	1 059	—	—	0.6	108	100	—	0.21
5	1 835	54.4	45.6	1.5	218	63.7	36.3	0.32
6	—	—	—	—	85	70	30	0.12

The absence of H_2S^{35} after large amounts of Na_2S^{35} have been injected into an organism can be explained in accordance with facts well-known in the literature [4, 5, 6, 7] of its oxidation and, to a lesser extent, its combination with organic compounds. From the subcutaneous tissue the H_2S^{35} slowly permeates into the blood where it is oxidized. From the blood the products of the transformation of H_2S pass into the tissues and are eliminated in the urine. In corroboration we adduce the results of an examination performed on 2 rats over a period of 6 days after a single injection of Na_2S^{35} (Table 1).

As is apparent from Table 1, the greatest amount of the injected S^{35} is eliminated the first day. Inorganic sulfates make up the greater part of the total sulfur. Elimination of S^{35} falls off sharply during the subsequent days. Prolonged elimination of small amounts of S^{35} apparently results from the distribution of S^{35} within the organism among other sulfur-containing metabolic products, together with which the S^{35} is gradually eliminated. It is possible, however, that part of the S^{35} is included in organic compounds and is eliminated from the organism only with their disintegration. In order to shed light on this problem a study was made of the distribution of S^{35} (per cent specific activity of S^{35} [2]) in the organs of the rat 1 day after (Experiment 1) and 6 days after (Experiment 2) a single injection of Na_2S^{35}.

TABLE 2

Specific Activity of S^{35} in the Organs of Rats

Organs	Experiment 1			Experiment 2		
	Na_2S^{35} was injected subcutaneously					
	468 γ (871 × 10^3 imp/min)			520 γ (968 × 10^3 imp/min)		
	sulfur in mg/g of tissue	imp/min per g of tissue	Spec. act. × 10^{-2}	sulfur in mg/g of tissue	imp/min per g of tissue	Specific activity, × 10^{-2}
Lungs	1.0	675	7.7	1.3	244	2.7
Liver	1.4	1 007	8.2	1.6	144	0.9
Kidneys	0.5	1 363	32.2	1.9	363	1.9
Spleen	1.7	2 742	19.5	1.4	294	2.2
Brain	0.6	418	7.7	0.7	197	2.7
Heart	1.8	514	3.2	2.2	132	0.6
Skeletal muscles	1.1	89	0.9	1.8	107	0.6

It is apparent from Table 2 that the greatest specific activity 1 day after injection of Na_2S^{35} is observed in the kidneys, then in the spleen and liver. This is explained by the removal by the kidneys of the products of the metabolism of H_2S. The least activity was detected in the skeletal muscles. Six days after injection of the sulfide the specific activity falls off considerably. The greatest activity is detected in the lungs and brain, the least in the muscles. Apparently the residual activity is due to a comparatively more stable connection of S^{35} with the organs. One may suppose that part of the S^{35} is included in the proteins of the organs.

TABLE 3

S^{35} Content in Protein Preparations of the Organs

Organs	Na_2S^{35} was injected subcutaneously		
	46 γ (3.5×10^6 imp/min)	72 γ (8.3×10^6 imp/min)	495 γ (11.1×10^6 imp/min)
	S^{35} content (no. of impulses/min in the heavy layer)		
Lungs	10	39	94
Liver	0	15	29
Kidneys	8	110	185
Spleen	4	41	76
Heart	2	14	33
Brain	3	14	74
Skeletal muscles	0	8	29

In order to shed light on this question a determination was made of the content of S^{35} in the protein preparations of organs. With the aim of increasing the amount of S^{35} injected into the organisms Na_2S^{35} was subcutaneously injected daily into rats for a period of 3-5 days. The determinations were carried out 24 hours after the last injection of the sulfide (Table 3).

It is apparent from Table 3 that, when the amount of sodium sulfide injected is increased, the S^{35} content in the protein preparations of the organs increases. The radioactivity connected with the proteins of the organs, however, proved to be very slight. We do not have data available on the nature of the connection of S^{35} with the tissue proteins. Apparently the presence of S^{35} in protein preparations of the organs cannot be explained by the simple adsorption of sulfates. In order to shed light on this question we treated the protein preparations of the organs for 2-24 hours at room temperature with a 0.001-0.003 M solution of Na_2SO_4 and examined the centrifugates. S^{35} was not detected in the centrifugates.

Our research confirms the fact that in the processes of the transformation of hydrogen sulfide within an organism the utilization of sulfur in the construction of organic compounds is of minor importance in comparison with the fundamental process, that of removal by the kidneys of the oxidation products of H_2S.

SUMMARY

White rats were subcutaneously injected with Na_2S^{35}. The content of hydrogen sulfide in the blood, organs and urine was determined chemically and radiometrically on the 1st, 2nd, and 6th day. The distribution of S^{35} was studied in dried preparations of organs, blood, urine and in the proteins of the organs.

No hydrogen sulfide was detected in the blood, urine and lungs. The greater part of S^{35} was removed from the organism with the urine in the first 24 ohours. The utilization of sulfur in the formation of organic compounds is of secondary importance in comparison with the removal of oxidation products of H_2S by the kidneys.

LITERATURE CITED

[1] A. I. Gunina and V. A. Tikhonravov, Farmakol. i Toksikol., Vol. 16, No. 6, pp 46-49 (1953).

[2] I. A. Oivin, Farmakol. i Toksikol., Vol. 13, No. 5, pp 45-48 (1950).

[3] I. A. Oivin et al., Byull. Eksptl. Biol. i Med., Vol. 15, No. 6, pp 44-47 (1943).

[4] I. A. Oivin et al., ibid., Vol. 16, No. 9, pp. 42-46 (1943).

[5] I. A. Oivin et al., ibid., Vol. 16, No. 10-11, pp. 41-43 (1943).

[6] I. A. Oivin et al., ibid., Vol. 16, No. 12, pp. 55-58 (1943).

[7] V. A. Tikhonravov and A. N. Iskova, Farmakol. i Toksikol., Vol. 4, No. 4-5, pp 25-28 (1941).

[8] D. D. Dziewiatkowski, J. Biol. Chem., Vol. 161, pp 723-729 (1945).

[9] L. H. Smith, B. Anderson, and T. T. Odell, Proc. Soc. Exptl. Biol. Med., Vol. 90, No. 2, pp. 360-364 (1955).

[10] H. Tarver and C. L. A. Schmidt, J. Biol. Chem., Vol. 130, pp 67-80 (1939).

TEMPERATURE CHANGES OF THE HUMAN SKIN IRRADIATED WITH LOW INTENSITY WAVES SEVERAL CENTIMETERS IN LENGTH

A. S. Pressman

From the Institute of Labor Hygiene and Occupational Diseases
(Director – Prof. A. A. Letavet, Regular Member of the Academy of Medical Sciences of the USSR) of the Academy of Medical Sciences of the USSR, Moscow

(Received March 23, 1955. Presented by Prof. A. A. Letavet, Regular Member of the Academy of Medical Sciences of the USSR)

In the last few years a number of works have been published which are devoted to the use of radio waves of the several-centimeter band in medicine and biology. It has been established that these waves are a more effective thermogenic agent than the ultra-short waves widely used in physiotherapy. Together with the thermal effect of waves of several centimeters in length the possibility is assumed of their extrathermal (specific) effect. But there are still too few experimental data to confirm this supposition.

One of the important tasks in studying the biological effect of the several-centimeter waves is an examination of the relationship between thermal effect and the intensity and duration of irradiation. In a great number of the published works, however, such examinations are more often of a qualitative nature. The intensity of the irradiation was only relatively determined – according to the power output of the generator and the distance from the object to the irradiator, while the temperature of the object was measured after irradiation ceased.

The most exact quantitative relationships were obtained in the experimental work of Seguin and Pelletier [4], Boyle et al., [2] and Cook [3].

Employing special apparatus and methods, these authors secured satisfactorily exact dosage of the wave intensity absorbed in the tissues of the object of irradiation as well as temperature measurement during the irradiation process. They established that in irradiation of a part of the human body according to their method the greatest temperature rise is noted on the surface of the skin.•

In our work we measured the skin temperature of a portion of the human body (the shoulder) irradiated with waves of the ten-centimeter band at low intensity in order to determine that minimal intensity at which a temperature increase is still not observed. The establishment of such a threshold intensity according to thermal effect is a matter of practical interest and is necessary in deciding the question of the presence of an extrathermal effect from the several-centimeter waves.

EXPERIMENTAL METHODS

With a few alterations the technique employed in our experiments was that of H. Cook.

Figure 1 contains a simplified diagram of the wave-conducting dosimeter. The several-centimeter waves created by the generator (1) radiate through the wave-conducting tract of the dosimeter which ends in an

• For the research methods and results, cf. A. Pressman, Uspekhi Sovremenoi Biol., Vol. 41, No. 1, pp 40-54 (1956).

irradiator horn (5) against which the object being irradiated (6) is pressed. The amount of power delivered to the object is regulated by means of a blade attenuator (2) which absorbs part of the power. Measurement of the power delivered to the object is accomplished with an indicator (3) through delivery of a negligible portion of the power from the wave-conducting tract to a power meter. A special adjusting device (4) compensates for the partial reflection of waves from the object being irradiated and thus guarantees its absorption of practically the entire power output ("adjustment" with the object).

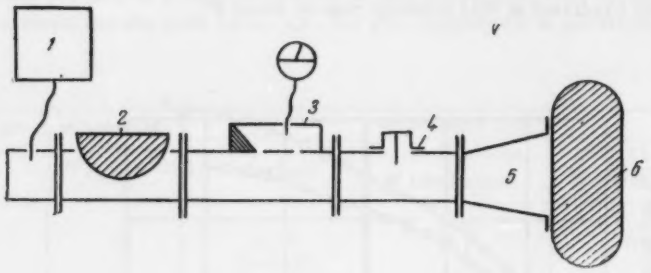


Fig. 1. A simplified diagram of the wave-conducting dosimeter.

The irradiator horn with a mouth area of 40 cm^2 has a flanged rim to which is attached a felt pad treated with aquadag. Such a pad secures good heat insulation of the portion of the body pressed to the rim of the horn from the colder surface of the horn flanges. Considered from another aspect, such a pad eliminates the necessity of pressing the part being irradiated hard against the horn, a practice which can lead to difficulty in blood circulation. Previous research has shown that even when the body part being irradiated is pressed very weakly against the horn through a pad not more than 1% of the power output is absorbed in it.

Measurement of the temperature in the center of the part being irradiated was accomplished with a thermocouple (copper–constantan) on a polystyrene plate fastened across the mouth of the horn perpendicular to the lines of electric force. The junction of the thermocouple was located in the center of the mouth of the horn. The accuracy of the temperature measurements amounted to 0.1°.

The power absorbed by 1 cm^2 of the surface of the object (we called this the "effective intensity") was determined for the central portion of the part being irradiated (where the temperature was measured) in mw/cm^2, with an accuracy of ± 11%. The irradiation was performed with sustained waves 11 cm in length.

The method of irradiation and temperature measurement was as follows:

a) After adaptation to the surrounding temperature the bare arm was placed on a special soft support so that the inner portion of the shoulder pressed lightly against the irradiator horn, thus guaranteeing close contact of the skin surface with the pad on the horn flange along its perimeter. Skin temperature was recorded (in the central portion of the part being irradiated) by means of the thermocouple galvanometer until it finally remained fixed.

b) During delivery of negligible power to the object the adjusting device was set to provide maximal absorption of the power output within the object.*

At the time of the adjustment an observation was made of the skin temperature.

c) After the adjustment the dose attenuator was set to deliver to the object a fixed power (registered on the meter), and the readings of the thermocouple galvanometer were recorded every minute.

The basic experiments were conducted at an air temperature of 19-21°. The subjects of the examinations in all of the experiments were men 28, 29 and 45 years of age.

* Control of the adjustment was accomplished on a guage line (not shown in Fig. 1) by measuring the coefficient of the running wave (CRW), which is characteristic of the degree of power absorption in the object.

RESULTS OF THE EXPERIMENTS

As was pointed out, the main purpose of the experiments was to examine the temperature changes of the skin irradiated with waves several centimeters in length in order to determine the minimal intensity which does not produce a thermal effect. It was therefore necessary, first of all, to select an approximate intensity, maximal for the given experiments. In choosing this intensity we proceeded from the experiments of Cook [3] in which the scarcely endurable intensity amounted to 600-800 mw/cm^2. We took 35 mw/cm^2 as our maximal intensity, a value about 20 times smaller than the mean scarcely endurable intensity. The experiments showed that the temperature of skin irradiated at that intensity rises by about 3°.

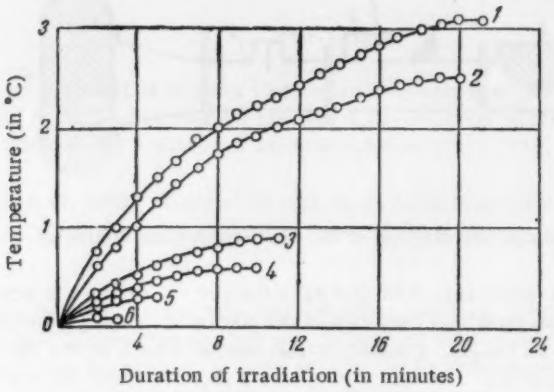


Fig. 2. Rise in temperature of the human skin (the shoulder) irradiated with several-centimeter waves (λ = 11 cm) in relation to duration and intensity of irradiation: 1) 35 mw/cm^2; 2) 25 mw/cm^2; 3) 20 mw/cm^2; 4) 15 mw/cm^2; 5) 10 mw/cm^2; 6) 5 mw/cm^2.

An examination was made of normal temperature reactions under the conditions of the experiments. It turned out that over the course of 30 minutes the temperature of the portion of the skin being examined varied within the limits of 0.1°.

A check was also made of the possible change in skin temperature at the time of the adjustment. Within the limits of accuracy of the measurements, no temperature change was detected.

It was also necessary to check whether the thermocouple was heated on account of direct absorption of wave energy.

The experiments showed that no such heating occurs.

In the first series of experiments an examination was made of the relationship between the temperature rise of the portion of the body being irradiated and the duration of irradiation at varying intensity. As is apparent from Fig. 2, according to the extent of irradiation the rise in skin temperature gradually attains a maximum value and is maintained at this level during further irradiation. It is known that the same phenomenon occurs with infrared radiation and is explained by the active incorporation of thermoregulating mechanisms which maintain heat exchange at a definite equilibrium level [1]. Evidently a similar equilibration mechanism takes place in the case being described. Since the maximal temperature rise was observed after approximately 22 minutes of irradiation at the maximal intensity (35 mw/cm^2), the duration of irradiation in all of the experiments amounted to 25 minutes.

In the second series of experiments the relationship between change in skin temperature and the average rate of maximal temperature elevation was determined for each intensity. A simultaneous comparison was made

of the values of the rise in temperature during irradiation at different intensities for periods of the same duration. The table contains the data of the experiments on irradiation at different intensities at an air temperature of 19-21°. Under these conditions the normal temperature of the portion of the skin of the shoulder under examination amounted to 32-33.9°.

A graph was constructed in accordance with the data of the table. Figure 3 shows how the maximal rise in skin temperature increases with augmentation of the effective intensity. It is evident from Fig. 3 and the table that at an effective intensity of 5 mw/cm^2 the rise in skin temperature does not exceed 0.1°, while the accuracy of the measurement has the same value; i.e., the skin temperature is practically not increased.

Effective intensity in mw/cm^2	Number of experiments	Temperature rise		
		Maximal	After 5 minutes of irradiation	After 11 minutes of irradiation
5	12	0.1° (0—0.2°)	0.1°	0.1°
10	14	0.3° (0.2—0.4°)	0.3°	0.3°
15	14	0.6° (0.5—1.0°)	0.4° (0.3—0.6°)	0.4°
20	13	0.9° (0.6—1.1°)	0.5° (0.4—0.6°)	0.9° (0.6—1.1°)
25	14	2.5° (2.3—2.8°)	1.0° (0.8—1.2°)	1.8° (1.6—2.1°)
35	10	3.1° (2.8—3.5°)	1.3° (1.1—1.5°)	2.3° (1.9—2.8°)

Note: The maximum and minimum values of the individual measurements are indicated in parentheses.

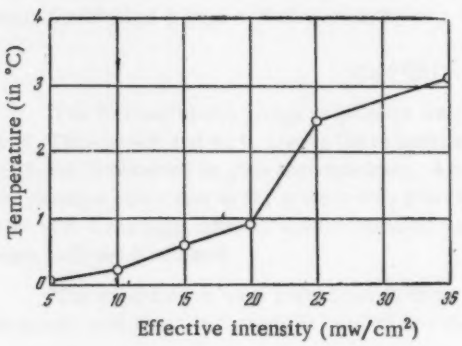


Fig. 3. Maximal rise in temperature of the human skin (the shoulder) irradiated with several-centimeter waves (λ = 11 cm) at different intensities.

On the basis of the results of the experiments the following conclusions can be drawn:

1) During irradiation of a part of the human body (the inner portion of the shoulder) 40 cm^2 in area with sustained several-centimeter waves (λ = 11 cm) the temperature of the skin increases gradually until reaching a definite (for a given intensity) maximal level and continues to maintain itself at this level during protracted irradiation. This equilibration is apparently explained by the incorporation of thermoregulation mechanisms which maintain heat exchange at a definite equilibrium level (as in the case of infrared radiation).

2) The maximal rise in skin temperature due to irradiation at an effective intensity (that absorbed in the tissues) of 35 mw/cm^2 amounts on the average to 3.1° (at an air temperature of 19-21°). According to the extent of reduction in intensity, the temperature rise grows steadily smaller, and at an intensity of 5 mw/cm^2 no rise in skin temperature occurs at all.

3) Under the experimental conditions described (air temperature, 19-21°; λ = 11 cm; surface irradiated, 40 cm^2) an effective intensity of 5 mw/cm^2 can be considered the threshold intensity for the thermal effect of several-centimeter waves.

SUMMARY

Heat effects (temperature rise of the skin) in the tissues of a skin area in man during irradiation with waves (λ = 11 cm) of different intensity (5 mw/cm^2 to 35 mw/cm^2) were studied.

Temperature rise of the skin was found to take place only in the case of the irradiation with waves of an intensity higher than 5 mw/cm^2. Temperature attains its maximum (at a given intensity) in 10 to 25 minutes of irradiation, remaining at this level afterwards.

LITERATURE CITED

[1] N. F. Galanin, Radiant Energy and its Hygienic Significance,* Leningrad (1952).

[2] A. C. Boyle, H. F. Cook, and T. I. Buchanan, Brit. J. Physiol. Med., Vol. 13, No. 1, pp 2-9 (1950).

[3] H. F. Cook, Journ. Physiol., Vol. 118, No. 1, pp 1-11 (1952).

[4] L. Seguin and M. Pelletier, Compt. rend. Acad. Sci., Vol. 226, pp 1048-1050 (1948).

* In Russian.

THE DISTRIBUTION OF FREE SULFHYDRYL GROUPS IN THE PROTEINS OF THE MUCOUS MEMBRANE OF DIFFERENT DIVISIONS OF THE STOMACH AND THE CHANGE IN THE AMOUNT OF THEM IN CONNECTION WITH THE SECRETION OF GASTRIC JUICE

E. E. Martinson and H. P. Lind

From the Department of Biochemistry of Tartu University

(Received August 10, 1956. Presented by Prof. A. E. Braunshtein, Regular Member of the Academy of Medical Sciences of the USSR)

In previous work on the functional biochemistry of the stomach we demonstrated the role of sulfhydryl groups in the biochemical mechanism of the secretory function of the stomach, the formation of hydrochloric acid and the neurohumoral regulation of this physiological process [3, 4, 5]. This was demonstrated by the reversible depression of hydrochloric acid formation through blocking with thiol inhibitors and removing the blocks with cysteine and by the direct stimulation of gastric secretion and hydrochloric acid formation through use of cysteine [5]. Since, aside from the soluble thiols glutathione and coenzyme A, the fundamental bearers of thiol groups are proteins, both enzymatic and structural protoplasmic proteins, the question naturally arose of the quantity of these most important reactive groups in proteins in the different sections of the mucous membrane of the stomach, i.e., in the proteins of its secretory apparatus, as well as the question of a change in content of sulfhydryl groups or their reactivity as a result of stimulation of gastric secretion.

EXPERIMENTAL METHODS

The free sulfhydryl groups of proteins were detected by Mirsky's ferricyanide method [7] as modified by A. S. Tsiperovich and A. L. Loseva [6] in homogenates of the stomach mucose (1 g of mucosa tissue + 9 ml 0.9% NaCl) obtained in glass homogenizers. After oxidation of the SH group by ferricyanide the intensity of the Prussian blue color in the protein-free filtrates was measured photometrically in a photoelectric colorimeter with a red light filter as against distilled water. The quantity of SH groups was expressed in microequivalents (μE) per g of tissue.

The experiments were performed on cats. The animals were exsanguinated via the carotid arteries; their stomachs withdrawn and carefully washed and the mucous membranes removed.

RESULTS OF THE EXPERIMENTS

In the first series of experiments after 24 hours without food, a determination was made of the content of sulfhydryl groups in the mucosa of the four divisions of the stomach: the cardia, the greater curvature, the lesser curvature and the pyloric portion (see Table).

As the table shows, most of the sulfhydryl groups are contained in the main and functionally the most active part, the body of the stomach, which secretes the acidic juice containing hydrochloric acid. Of the two divisions of the body of the stomach, the greater and lesser curvatures, in the state of functional rest, the quantity of SH groups is higher in the greater curvature

Quantity of Sulfhydryl Groups in μE per g of Stomach Mucous Membrane in the Resting State and During Secretion Induced by Histamine

No. of the Experiment	Cardia		Greater curvature		Lesser curvature		Pyloric portion	
	At rest	After injection of histamine	At rest	After injection of histamine	At rest	After injection of histamine	At rest	After injection of histamine
1	6.1	7.5	7.6	7.8	6.5	6.8	5.8	6.0
2	4.6	7.6	7.3	8.3	8.3	9.1	5.7	6.7
3	6.7	7.4	8.0	12.0	4.6	7.6	6.5	6.6
4	6.6	6.9	7.5	9.0	5.8	7.6	4.0	6.6
5	6.6	6.7	8.4	10.5	6.7	8.0	5.0	6.4
6	6.5	7.7	7.5	8.1	4.5	8.0	4.4	6.5
7	4.0	5.8	8.4	9.0	—	7.0	6.6	5.5
8	—	6.0	8.6	8.9	—	8.4	5.9	5.8
9	—	—	8.0	8.0	—	7.6	3.6	5.0
10	—	—	5.6	6.7	—	—	5.1	3.0
11	—	—	6.7	—	—	—	6.6	—
12	—	—	6.0	—	—	—	3.5	—
Average	5.9	6.8	7.5	8.8	6.1	7.8	5.2	5.8
increase in %		15.2		17.3		27.9		11.5

The least amount of sulfhydryl groups is contained in the pyloric portion, which secretes the juice of an alkaline reaction that does not contain hydrochloric acid.

Thus the distribution of sulfhydryl groups in the proteins of the secretory apparatus of the different divisions of the stomach corresponds to their functional secretory properties and to the significance of these groups in gastric secretion and the formation of hydrochloric acid [3, 4, 5].

When the mucous membrane was taken for analysis its delimitation into four divisions was not completely exact, and the differences in quantity of sulfhydryl groups were therefore leveled off to a considerable extent.

If the secretory function of the stomach is in fact linked with sulfhydryl groups, then an increase in the amount of them was to be expected with the stimulation of secretion. In the second series of experiments, determinations were made of the sulfhydryl groups in cats following prior subcutaneous injection of histamine at the rate of 1 ml per kg of body weight. Fifteen minutes after the injection the stomachs of the animals were taken for analysis. The data of these experiments are also included in the table.

As is apparent from the table, the change-over of the secretory apparatus of the stomach from the state of rest to physiological activity is accompanied by an increase in the amount of free sulfhydryl groups of the protein substances. The greatest increase occurs in the body of the stomach, the least in the pyloric portion. It is interesting to note that in the lesser curvature the accumulation of sulfhydryl groups is significantly greater than in the greater curvature.

According to the data of K. M. Bykov's laboratory [1], the juice released by the lesser curvature has the highest acidity. According to K. M. Bykov, a leading role in the development of the secretory process of the entire stomach belongs to the lesser curvature.

Yu. M. Lozovsky [2] showed that the most marked changes in structure during secretion occur in the glandular cells of the lesser curvature of the stomach.

Thus the characteristic topographic distribution of SH groups in the mucosa of the stomach and the change in the quantity of them in connection with secretion show that in the development and accomplishment of the physiological act of secretion the structure of the protein substance of the secretory apparatus is of considerable importance. To all appearances secretion is linked with a change in the structure of the proteins, as a result of which there is a change in the number of their free sulfhydryl groups, which activate the biochemical processes that cause the physiological act of secretion. The question still remains open concerning the extent to which

there occurs an actual increase in the number of SH groups and in their reactivity in a given instance of oxidizability. It is also possible that the characteristic distribution of sulfhydryl groups reflects a difference in the structure and properties of the proteins of the secretory apparatus of the different divisions of the stomach. This idea provides a biochemical basis for K. M. Bykov's conception of the secretory fields of the stomach. There is a need for study of the proteins of the mucous membrane of the stomach in connection with its secretory function.

SUMMARY

The quantity of free SH-groups in the proteins of the stomach mucous membrane is differently distributed in the divisions of this organ: the smallest amount of these groups is to be found in the pyloric portion, whereas the maximal content is characteristic of the greater and lesser curvatures. Secretion being stimulated by means of histamine, the quantity of SH-groups increases particularly in the lesser curvature and least in the pyloric region.

LITERATURE CITED

[1] I. T. Kurtsin, The Physiology of Digestion,* Leningrad (1954).

[2] Yu. M. Lozovsky, The Normal and Pathological Functional Morphology of the Stomach,* Moscow (1947).

[3] E. E. Martinson and H. Lind, Byull. Eksptl. Bio. i Med., No. 11, pp 21-24 (1952).

[4] Ibid., Vol. 40, No. 7, pp 20-23 (1955).

[5] E. E. Martinson and H. Lind, in the book: Abstracts of Papers Presented at the Eighth All-Union Convention of Physiologists, Biochemists and Pharmacologists in Kiev,* Moscow, pp 399-400 (1955).

[6] A. S. Tsiperovich and A. L. Loseva, Ukrain. Biokhim. Zhur., Vol. 20, No. 1, pp. 94-107 (1948).

[7] A. E. Mirsky, J. Gen. Physiol., Vol. 24, No. 6, pp 709-723 (1941).

* In Russian.

PHARMACOLOGY

THE GANGLIOPLEGIC PROPERTIES OF AMINAZINE (LARGACTIL) AND MEPAZINE (PACATAL)

D. A. Kharkevich

From the Laboratory of Special Pharmacology (Director – Prof. V. V. Zakusov, Regular Member of the Academy of Medical Sciences of the USSR) of the Institute of Pharmacology and Chemotherapy of the Academy of Medical Sciences of the USSR, Moscow

(Received July 11, 1956. Presented by V. V. Zakusov, Regular Member of the Academy of Medical Sciences of the USSR)

In studying the pharmacological properties of largactil (aminazine) Laborit and his co-workers [12, 13] came to the conclusion that this compound possesses gangliolytic properties. Many other authors [1, 3, 4, 5, 9, 17] hold the same viewpoint. At the same time there appeared in the literature reports,denial of the ability of largactil to depress the transmission of stimulation in the autonomic ganglia [6, 7, 8, 16]. General opinion holds this question still open to debate [10, 11, 14, 15].

The aim of the present research is to clarify whether largactil influences the transmission of stimulation in the superior cervical ganglion of the cat.

EXPERIMENTAL METHODS

In experiments on decerebrated cats simultaneous recording was maintained of the biopotentials of the postganglionic fibers and the contractions of the nictitating membrane. The preganglionic fibers were irritated with rectangular stimuli with a frequency of 20-40 cps. The duration of each impulse was 0.1-0.5 milliseconds. The substances were injected intravenously.

The choice of such a method was occasioned by the fact that the more widespread means of testing ganglioplegic agents cannot always be used in a given instance. Thus, to draw conclusions about the ganglion blocking activity of largactil according to the reaction of the effector organ (nictitating membrane, pupil, the action of the heart, etc.) arising in response to irritation of preganglionic fibers is difficult and at times simply impossible (when the substance is injected into the general blood stream). This pertains to both sympathetic and parasympathetic innervation and is caused by the ability of largactil to exert an atropine-like adrenolytic and sympatholytic effect. More expedient for such examinations is the oscillographic method, which permits the derivation of biopotentials directly from the postganglionic fibers or the ganglion while the blood supply of the ganglionic cells is maintained.

RESULTS OF THE EXPERIMENTS

Largactil in a dose of 2.5-5 mg/kg of body weight completely prevented the reaction of the nictitating membrane to irritation of the preganglionic trunk. As is shown in Fig. 1, in this case the biocurrents not only did not decrease, but even increased somewhat in the given experiment. The oscillogram in Fig. 1c, serves as evidence that the substance reaches the cells of the ganglion. The complete absence of biocurrents on it is linked with the use of tetraethylammonium (10 mg/kg), which blocked the transmission of stimulation in the ganglionic synapses. The negligible lowering of biocurrents which was observed in individual cases when large

doses of largactil (of the order of 10-20 mg/kg) were used can be explained by the disturbance to the blood supply of the ganglionic cells in connection with the falling off of blood pressure.

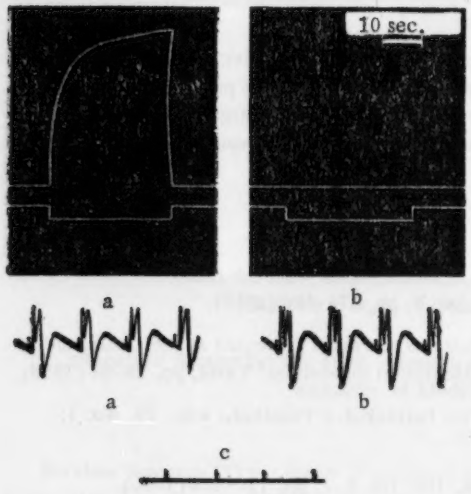


Fig. 1. Simultaneous recording of the contractions of the nictitating membrane and the biocurrents of the postganglionic fibers during electrical irritation of the preganglionic trunk. a) before injection of largactil; b) after injection of largactil, 2.5 mg/kg; c) a minute after injection of tetraethylammonium, 10 mg/kg. Frequency of irritation: 20 cps. Duration of each stimulus: 0.5 milliseconds. Amplitude 1 v. The short vertical lines on the oscillograms are the artifacts of the irritation. The biocurrents corresponding to the mechanograms are designated with the same letters.

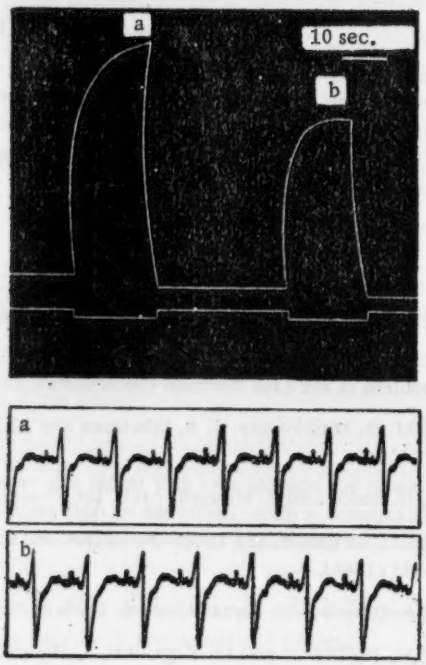


Fig. 2. Simultaneous recording of the contractions of the nictitating membrane and the biocurrents of the postganglionic fibers during electrical irritation of the preganglionic trunk. a) before injection; b) after injection of pacatal, 5 mg/kg. The other designations are the same as in Fig. 1.

Thus the absence of a reaction on the part of the nictitating membrane while the amplitude of the biocurrents remains unchanged attests to the peripheral sympatholytic effect of largactil. Transmission of impulses in the ganglion in this case is not depressed.

Analagous data were obtained also for the other representative of the phenothiazine series, mepazine (pacatal), which was synthesized in the chemistry department of the Institute of Pharmacology and Chemotherapy of the Academy of Medical Sciences of the USSR by S. V. Zhuravlev, A. N. Gritsenko and M. I. Dorokhova. The pharmacology of this agent has been studied by Yu. I. Vikhlyaev [2]. In a dose of 5-10 mg/kg, pacatal as a rule does not exert a depressant influence on the cells of the superior cervical ganglion, a fact which may be inferred from the absence of changes in the biopotentials of the postganglionic fibers (Fig. 2). The reaction of the nictitating membrane following the use of pacatal decreased to a lesser extent than from the use of largactil. This can be perceived in a comparison of Fig. 1 and Fig. 2. Largactil in a dose of 5 mg/kg usually completely prevented contraction of the nictitating membrane, while pacatal in a dose of 5-10 mg/kg decreased it on the average by 10-30%.

Consequently the sympatholytic properties of pacatal are less marked than those of largactil.

The data obtained attest to the fact that largactil and pacatal in so-called therapeutic doses do not exert

a depressant influence on the transmission of stimulation in the superior cervical ganglion. The weakening of the contractions of the nictitating membrane which is observed when these substances are used is the result of their symatholytic effect. Thus one may conclude that largactil and pacatal do not possess ganglioplegic properties.

SUMMARY

The preganglionic sympathetic trunk of the cat was stimulated by rectangular electric shocks of 20 cps frequency, and the contractions of the nictitating membrane and the biocurrents of the postganglionic fibers of the superior cervical ganglion were simultaneously recorded: neither aminazine (largactil) nor mepazine (pacatal) in doses of 5-10 mg/kg exerts any ganglioplegic effect. Sympatholytic properties of aminazine are more pronounced than those of mepazine.

LITERATURE CITED

[1] I. P. Anokhina, Zhur. Nevropatol. i Psikhiat., vol. 56, No. 6, pp. 478-488 (1956).

[2] Yu. I. Vikhlyaev and G. I. Chugunov, in the book: Abstracts of Papers Presented at the Conference on the Problem of the Link between the Structure and Effect of Medicinal Substances,* Tartu, pp. 23-24 (1956).

[3] M. D. Mashkovsky, S. S. Liberman and A. I. Polezhaeva, Farmakol. i Toksikol., vol. 18, No. 1; pp. 14-22 (1955).

[4] H. Budde and E. Witzleb, Arch. int. pharmacodyn., vol. 102, No. 1-2, pp. 126-138 (1955).

[5] S. Courvoisier, I. Fournel, R. Ducrot, M. Kolsky, and P. Koltcshet, Arch. int. pharmacodyn, vol. 92, pp. 305-361 (1955).

[6] P. Decourt, M. Brunaud, and S. Brunaud, Compt. rend. soc. biol. vol. 147, pp. 1602-1605 (1953).

[7] M. Holzbauer and M. Vogt, Brit. J. Pharmacol., vol. 9, pp. 402-407 (1954).

[8] F. Huidobro, Arch. int. pharmacodyn, vol. 98, No. 3, pp. 308-319 (1954).

[9] H. Kewitz, Arch. exp. path. u. pharmakol., Bd. 222, S. 323-329 (1954).

[10] H. Kopera, Wien. klin. Wschr., 67, N. 45, S. 867-874 (1955).

[11] Idem. Ibid, No. 46, S. 887-892, (1955).

[12] H. Laborit and P. Huguenard, Presse med., ann. 59, No. 64, pp. 1329-1329, (1951).

[13] H. Laborit, P. Huguenard, and P. Alluame, Presse med., No. 10, pp. 206-208 (1952).

[14] O. Nieschulz, R. Popendiker and I. Hoffmann, Arzneimit.-Forsch., No. 2, S. 680-695 (1955).

[15] A. Quevauviller, Produits pharm., vol. 10, No. 3, pp. 175-182 (1955).

[16] I. I. Reuse, Compt. rend. soc. biol. vol. 148, pp. 192-193 (1954).

[17] H. Ruppert, Zbl. Gynakol., 77, No. 7, S. 250-256 (1955).

* In Russian.

THE FATE OF SYNTOMYCINE AND ITS OPTICAL COMPONENTS IN ANIMAL ORGANISMS

G. N. Pershin and V. V. Nesvadba

From the All-Union Chemical and Pharmaceutical Research Institute
(Director – M. V. Rubtsov, Doctor of Chemistry), Moscow

(Received September 28, 1956. Presented by V. N. Chernigovsky, Regular Member of the Academy of Medical Sciences of the USSR)

Previous research [1] has shown that after a single injection of a rabbit with syntomycine the preparation is accumulated in almost all of the organs in greater concentrations than in the blood. With a comparatively low syntomycine content in the blood, amounts of it capable of securing a medicinal effect may be accumulated in the tissues.

The aim of the present work was to examine the distribution of the optical components of syntomycine (L-mycetine and D-mycetine) and their capacity for being accumulated in the organs and tissues.

The distribution of D-mycetine and L-mycetine in an organism was studied in rabbits by a method which has been previously described [1]. The results of the experiments are set forth in Table 1.

TABLE 1

Content of Syntomycine, L-Mycetine and D-Mycetine in the Fluids and Tissues of the Rabbit 4 hours After Single Injection of the Preparation in the Form of an Aqueous Suspension

Weight of the rabbit in g	Preparation	Dose per animal in g	Content of the preparation in μg per g or per ml							
			Blood	Card. muscle	Skeletal muscle	Brain	Spleen	Liver	Kidneys	Lung
2400	Syntomycine	0.4	7.2	13	17	9	30	39	108	562
2415		0.4	18	24	12	15	28	49	138	104
2410		0.48	21	37	20	19	46	89	204	504
2600		0.52	18	35	16	< 10	22	98	213	92
2150	L-Mycetine	0.35	18	19	17	7	40	60	205	47
2185		0.36	18	19	12	32	38	41	276	39
2450		0.49	37	31	23	14	40	74	273	70
2500		0.5	15	18	< 10	< 10	42	54	198	28
2450	D-Mycetine	0.49	25	29	15	22	51	88	222	130
2450		0.49	19	24	23	20	52	58	99	52
2530		0.5	40	47	27	30	28	98	312	124

As is shown in Table 1, L-Mycetine and D-mycetine, like syntomycine, are accumulated in certain tissues and organs in greater concentrations than in the blood. The spleen, brain, liver, kidneys, cardiac muscle and skeletal muscles contain approximately the same amount of the preparations regardless of whether the animal received syntomycine, D-mycetine or L-mycetine. In our experiments the tendency to be accumulated in the lungs was somewhat more strongly marked with syntomycine.

We also examined the dynamics of the elimination of syntomycine from an organism (Table 2).

Table 2 shows that after a single injection of syntomycine (0.2 mg per g of body weight) the preparation is detected in a rabbit's urine for 2-3 days; during this period from 3.5 to 7% of the dose of syntomycine injected into the rabbit is eliminated in the urine.

TABLE 2

Elimination of Syntomycine from the Rabbit after a Single Injection of the Preparation in a Dose of 0.2 mg per g of Body Weight

Rabbit's weight in g	Dose of the preparation per animal	Amount of the preparation eliminated, in mg										Total amount of the preparation eliminated			
		1st day		2nd day		3rd day		4th day		5th day		in the urine		in the feces	
		in the urine	in the feces	in the urine	in the feces	in the urine	in the feces	in the urine	in the feces	in the urine	in the feces	in mg	in % of the amount injected	in mg	in % of the amount injected
2790	0.55	26.2	1.73	9.3	0.43	2.9	3.12	—	1.46	—	—	38.4	7	6.74	1.22
2810	0.55	0.42	0.1	27.48	0.8	1.67	3.23	—	0.70	—	—	29.57	5.4	4.83	0.88
1990	0.40	12.45	5.9	1.43	1.76	—	2.9	—	1.4	—	1.1	13.88	3.5	12.7	3.1
2200	0.44	18.08	18.4	2.67	1.78	—	1.72	—	1.4	—	0.5	20.75	4.7	23.8	5.4

A small amount of syntomycine can be detected in the feces of a rabbit on the fourth and fifth days; from 0.88 to 5.4% of the dose of syntomycine received by a rabbit is eliminated in the feces. In all, 6-10% of the injected dose of syntomycine is eliminated in the urine and feces. Not less than 90% of the preparation is subjected to considerable transformation within the organism.

SUMMARY

Like syntomycine, its optical components (L-mycetine and D-mycetine) are accumulated in many tissues and organs in a higher concentration than in the blood.

The study of the elimination of syntomycine from organisms has shown that 6 to 10% of the preparation leaves the organism in the urine and feces. Not less than 90% of the preparation is considerably transformed within the animal organism.

LITERATURE CITED

[1] G. N. Pershin and V. V. Nesvadba, Chemistry and Medicine,* p. 31, State Medical Press, Moscow (1954).

* In Russian.

MICROBIOLOGY AND IMMUNITY

RELATION BETWEEN THE LEVEL OF SPECIFIC ANTITOXIN IN THE BLOOD AND THE DEGREE OF IMMUNITY TO TETANUS TOXIN IN WHITE MICE IMMUNIZED WITH ADSORBED TETANUS TOXOID

A. A. Vorobyev, B. S. Goncharov, L. P. Lubyansky and A. V. Markovich

The Naval Medical Academy, Leningrad

(Received June 7, 1956. Presented by Member of the Academy of Medical Sciences of the USSR, P. F. Zdrodovsky)

It is generally agreed that the antibody level of the blood does not reflect completely the state of immunity of the organism as a whole, although the relation between humoral immunity and general immunity has been little studied. Only scattered observations relating to this problem are available [1, 3, 4].

In view of this we studied, in white mice (weight 17-20 g) immunized by a single intra-abdominal injection of purified tetanus toxin adsorbed on aluminum hydroxide (series 10 k), the content of the blood of specific antitoxin and the degree of immunity to tetanus toxin.

EXPERIMENTAL METHODS

We took blood for the determination of antitoxin from the tail veins, by shaving and cutting off a piece 2-3 cm long (beforehand we put the tail in warm water). We obtained about 5 drops of blood from each mouse; to avoid later loss of blood we attached a ligature. The determination of antitoxin was repeated 3-4 times (the average values are presented) by the usual method [6] on the pooled serum of that group of mice which had been immunized with the same dose of antigen.

We determined the degree of immunity to tetanus toxin by the method previously described by us [2]. As a quantitative measure of the degree of immunity of a group of white mice we used the LD_{50} (which is the dose of tetanus toxin, in MLD_{100}, , leading to the death of 50% of the mice), which was calculated by the probit method [5] by accumulating deaths and survivals by the method of Reed and Muench.

As a preliminary we examined the influence of blood taking on the degree of immunity to tetanus toxin. In this experiment we immunized 77 mice with 0.05 ml of antigen. After about 21 days the first group (40 mice not bled) had an LD_{50} of 229 MLD_{100} while in the second group (37 mice with preliminary bleeding) the LD_{50} was 174 MLD_{100} of toxin. Thus a preliminary bleeding somewhat lowers the degree of immunity of the mice.

RESULTS OF THE EXPERIMENTS

In Table 1 are presented the changes in average antitoxin levels (AE) in the serum and the degree of immunity to tetanus toxin LD_{50} in relation to the strength of the antigenic stimulus.

As is seen from Table 1, with increasing doses of antigen the AE and LD_{50} increase. But with increasing strength of antigenic stimulus the rate of antitoxin production falls behind the rate of increase in degree of immunity to toxin. Thus, on increasing the dose of antigen 2-fold (from 0.005 to 0.01 ml) the LD_{50} increases 4-fold, but the AE 3-fold; on increasing the dose of antigen 20-fold (from 0.005 to 0.1 ml) the LD_{50} increases 1780-fold, but the AE only 29.6-fold.

TABLE 1

Level of Antitoxin in the Blood and Degree of Immunity to Tetanus Toxin in White Mice about 21 Days after Immunization with Adsorbed Tetanus Toxoid

Dose of toxoid (ml)	Immunity to toxin			Average level of antitoxin in AE/ml
	Dose of toxin (MLD_{100})	Number of mice*	LD_{50} in MLD_{100}	
0.005	1	5/10	1	0.0025
	2	4/11		
0.010	1	6/2		
	5	5/3	4	0.0075
	10	1/6		
0.025	5	10/0		0.035**
	10	9/1	28,8	0.042
	20	8/2		
0.050	50	5/1		
	100	4/2	158	0.062
	200	3/2		
0.100	500	10/0		
	1000	9/1	1780	0.074
	2000	3/6		

* In the numerator, the number of survivors, in the denominator, the number of deaths with symptoms of tetanus within a week after the administration of the toxin.

** Result of another experiment set up a month before the main experiment.

In the following experiment, using another group of mice, we studied the change of AE and LD_{50} dynamics of immunity. The results obtained are given in Tables 2 and 3.

TABLE 2

Degree of Immunity to Tetanus Toxin in White Mice at Various Intervals after a Single Immunization with Adsorbed Tetanus Toxoid

Dose of toxoid (ml)	Days after immunization							
	7		14		22		28	
	Dose of toxin (MLD_{100})	No. of mice	Dose of toxin (MLD_{100})	No. of mice	Dose of toxin (MLD_{100})	No. of mice	Dose of toxin (MLD_{100})	No. of mice
0.01	1	2/13	1	8/2	1	8/0		
	2	0/15	5	7/3	5	5/4	5	4/3
			10	4/5	10	4/4	10	3/4
0.05	1	6/4	10	10/0	50	10/0		
	2	1/9	20	9/1	100	9/0	200	6/1
	5	0/10	50	7/2	200	8/1	400	5/3
	10	0/10	100	5/4	400	6/3	800	4/3
0.25	1	9/1	100	10/0	1000	9/0	2000	6/1
	2	4/6	200	10/0	2000	7/2	4000	5/2
	5	0/10	400	9/1	4000	5/3	8000	4/3
	10	0/10	800	6/3	8000	3/5	12 000	3/5

Note: In the columns headed "Number of mice" the numerator and denominator have the same significance as in Table 1.

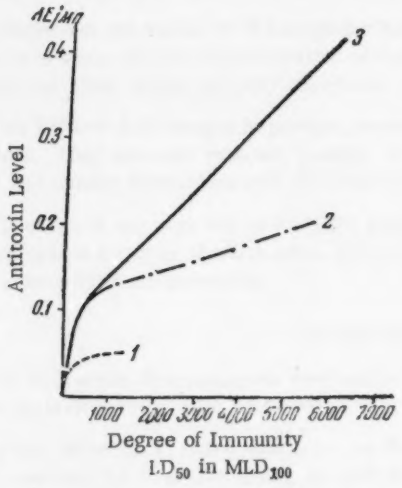
TABLE 3

Dynamics of Antitoxin Levels in the Blood (AE/ml) and Degree of Immunity (LD_{50}) Tetanus Toxin in White Mice Given a Single Immunization with Adsorbed Tetanus Toxoid

Doses of toxoid (ml)	Days after immunization							
	7		14		22		28	
	AE/ml	LD_{50} in MLD_{100}	AE/ml	LD_{50} in MLD_{100}	AE/ml	LD_{50} in MLD_{100}	AE/ml	LD_{50} in MLD_{100}
0.01	<0.0025	0.6	0.011	6.9	0.014	7.6	0.006	7.2
0.05	<0.0025	1.2	0.044	95	0.117	470	0.145	600
0.25	<0.0025	1.7	0.053	960	0.190	5000	0.395	6600

The results in Table 3 show that the more marked increase of the LD_{50} compared to the AE on increasing the dose of antigen, noted in the previous experiment is observed at all the stages of immunization investigated.

However the relation between the LD_{50} and the AE in the dynamics of immunity is more complicated. Even seven days after a single administration of 0.05-0.25 ml of adsorbed antigen, white mice already have acquired a measurable resistance to tetanus toxin (LD_{50} = 1.2-1.7 MLD_{100}), but at that time the antitoxin content of the blood is at about the limit of sensitivity of the method of determination (≤ 0.0025 AE). Between 14 and 22 days the relative increase in LD_{50} following immunization with 0.05 and 0.25 ml toxoid outstrips the increase of blood AE 1½ to 2-fold, while between 22 and 28 days the relative increase in the LD_{50} was the same (for 0.05 ml) of less (for 0.25 ml) than the relative increase in AE.



Dependence of degree of immunity of white mice to tetanus toxin on the level of specific antitoxin.
1) Interval after single immunization 14 days; 2) 22 days; 3) 28 days.

Thus after immunization with one and the same dose of antigen the relation between the relative tempo of increase in LD_{50} and AE changes during the development of immunity. From this it follows that the relation between the LD_{50} and the AE ought to change during the process of immunization. In the figure is shown the relation between the LD_{50} and the AE at various stages in the development of immunity.

As can be seen from the figure, a given LD_{50} corresponds to an AE which is larger the longer the interval after the single immunization.

Thus our experiments to study in the same white mice the average levels of antitoxin in the serum and the degree of immunity to tetanus toxin show that the increase in specific resistance of the whole organism accompanies an increase of antitoxin in the blood. But the laws governing the growth of antitoxic and general immunity, both in the dynamics of growth and in their dependence upon the strength of the antigenic stimulus, are different. The relation between the degree of immunity to toxin and the level of specific antitoxin in the blood changes with the dose of antigen and the time after the immunization. All this leads to the idea that the concentration of antitoxin in the blood is determined not only by the immune state of the organism, but also by other mechanisms regulating the appearance of antitoxin in the circulation.

SUMMARY

Levels of the specific antitoxin (AE) in blood and the immunity intensity against tetanus toxin (LD_{50}) were simultaneously studied on white mice immunized with the adsorbed tetanus toxic. The blood for the antitoxin determination was taken from the blood vessels of the tail (cut off): AE and LD_{50} increased with an increase of the antigen dose, the growth rate of the latter being much greater. The ratio between the growth rates of AE and LD_{50} changed in the course of the development of immunity.

LD_{50} being constant, AE level in the blood corresponded to the period elapsed after the antigen injection.

LITERATURE CITED

[1] A. Ya. Alymov and D. F. Pletsity, in the book: The Problem of Reactivity in Pathology,* (Moscow) 1954, pp. 9-18.

[2] A. A. Vorobyev, Byull. Eksptl. Biol. i Med. 33,43-47, No. 9, 1952.

[3] D. G. Manolov, Proceedings of the 6th Soviet Anaerobic Conference,* (Moscow) 1941, pp. 19-21.

[4] G. V. Shumakova, Problems of Infectious Pathology and Immunology,* (Moscow) 1954, pp. 127-136.

[5] C. Bliss, Ann. Appl. Biol. 22, 134, No. 1, 1935.

[6] G. Istrati, Zentralbl. f. Bakteriol. 143, 106-119, H. 1, 1938.

* In Russian.

CHANGE IN THE ANTIGENIC COMPOSITION OF CERTAIN PARENCHYMATOUS ORGANS IN WHITE MICE INFECTED WITH THE AGENT OF DYSENTERY AND STREPTOCOCCI

M. V. Zemskov

Voronezh State Medical Institute

(Received July 21, 1956. Presented by Member of the Academy of Medical Sciences of the USSR, N. N. Zhukov-Verezhnikov)

Reports which have recently appeared [1, 2, 3] on the discovery of foreign tissue antigens in a series of pathological processes present great practical and theoretical interest. The formation in the organism of foreign antigen indicates a marked change in protein metabolism due to a far-reaching pathological process. It is not excluded that such antigens may act as the source of pathological reflexes, maintain, exacerbate and complete new symptomatic and pathological bases for disease. Treatment of such a disease which takes into consideration only the original first cause cannot be successful.

Therefore, the discovery of foreign antigens might change our concepts of the pathogenesis of such diseases, or of some of them; determination of the character of the newly formed antigens, and means of rendering them harmless, might radically change the treatment of such diseases.

We believe that changes in protein metabolism are observed in various pathological processes, including infections. They are more extreme, possibly, in prolonged chronic processes, being accompanied by trophic lesions and lending themselves with difficulty to etiotropic treatment.

The aim of our work was to study the possibility of the formation of foreign antigens in the tissues of the liver, spleen, and kidney of white mice, infected separately with sublethal doses of the agent of Flexner dysentery and hemolytic streptococcus.

EXPERIMENTAL METHODS

In all, 8 series of experiments were carried out with guinea pigs. Foreign antigens were demonstrated by the anaphylaxis reaction with desensitization by L. A. Zilber's method.

From white mice, killed with ether, on the 5th day after infection (in one series after 24 hours) we aseptically removed the liver (discarding the gall bladder), the spleen, and kidneys. We ground up the organs finely in a mortar, suspended them in physiological saline, centrifuged the suspension at 2500 rpm. Under the skin of guinea pigs we injected about 1.5 ml of the supernatant liquid, containing from 26.4 to 13.2 mg % protein in 1 ml. After about 21-30 days all guinea pigs of the first 6 series were injected intracardially two or occasionally three times with the fluid corresponding to the organs of healthy mice, at intervals of $1\frac{1}{2}$ to 2 hours in doses of 0.2, 0.8 and 1 ml, with the aim of completely desensitizing the animals to the antigens of healthy mice.

About $1\frac{1}{2}$ - 2 hours after this we injected, also intracardially, each guinea pig with the original sensitizing material, in a dose equal to that used in the last desensitizing injection, after this we recorded the severity of the anaphylactic response.

Each series of experiments consisted of two variants, and in each variant 4 guinea pigs of about the same weight and the same color.

In the first variant we sensitized the guinea pigs with a suspension from the organs of infected mice with the aim of detecting the formation in them of new antigens; in the second variant the sensitization was carried out with a suspension of the organs of healthy mice with the aim of testing the possibility of the destruction in some of them of contained antigens (or antibodies). In this variant the desensitization was carried out with a suspension of the corresponding organ of infected mice, and the final reaction with a suspension of healthy organ.

In each series of experiments there were controls consisting of 3 guinea pigs. One guinea pig, to exclude the possibility of anaphylaxis due to the agent used to infect the mice, received a subcutaneous injection of a culture of the latter in a dose of 1-2 billion. After 21-30 days the same culture was injected intracardially twice, in increasing doses at intervals of $1\frac{1}{2}$ - 2 hours.

Into the remaining two unsensitized guinea pigs we injected, at the same intervals as those employed for the experimental animals, the corresponding organ suspension to test for toxic properties.

The experiments were carried out by the assistants of our department, E. D. Zhuravlevaya and N. K. Bondarenko, also by V. N. Tarasov and A. M. Gnetnev.

RESULTS OF THE EXPERIMENTS

The results of the experiments given in Table 1 reflect in general the final anaphylactic reaction obtained with the original sensitizing material.

From Table 1 it is evident that the control was in all cases unimpeachable.

The experiments of Series I show a formation of foreign antigens, and a destruction of a certain amount of antigens present, in the livers of mice infected with the agent of dysentery and killed after 24 hours. The experiments of Series II support these findings and at the same time show a direct relation between the duration of the process of dysentery infection in mice and the extent of formation of foreign antigens in their tissues. The data of Series III and IV show the regularity of the formation of foreign antigens in the organism of the mouse infected with dysentery bacilli, it being known that the greatest amount of these antigens are formed in the spleen and kidneys.

In the livers of mice infected with streptococci there is formed a little new antigen and a certain amount of antigen present is destroyed (Series V).

In the spleens of mice similarly infected there is formed a significant amount of foreign antigens and a certain amount of antigen present is destroyed.

Between the degree (strength) of anaphylaxis and the amount of foreign antigen formed there is no correspondence, however there is a close connection, which we took into account in the interpretation of our experiments.

It might be expected that in mice with the most prolonged and severe infections the greatest amount of foreign antigens would accumulate and the anaphylactic reaction in guinea pigs would be stronger and more marked.

In every case of dysentery and streptococcal infection of mice, foreign tissue antigens were formed and there was some, although not significant, destruction of the antigens present; this fact must influence the course of the corresponding infection. It is very probable that the formation of foreign tissue antigen constitutes a fourth indefeasible factor of each chronic infection.

It is very important to know whether specific foreign antigens are formed in the various organs, but from different infections, that is, to what extent there is a universal reaction of the foreign antigens formed.

With this aim Series VII and VIII of experiments were set up with 4 guinea pigs in each series.

Guinea pigs were sensitized with a suspension of the spleen of mice infected with the agent of Flexner dysentery (Series VII) and hemolytic streptococcus (Series VIII) and killed after 5 days. The pigs were desensitized to the antigens of the spleen of healthy mice, after which two of each series received the original

sensitizing material (direct reaction), and the remainder heterologous material (cross reaction). The results of the experiment are shown in Table 2.

TABLE 1

Formation of Antigens in Organs of Infected Mice

Series	Sensitizing Material	Number of desensitized guinea pigs	Final reaction with original sensitizing material			
I	Liver of mice: 24 hours after dysentery infection	4	—	+	+++	+
	Healthy	4	+	+	—	+
	Control	3	—	—	—	
II	Liver of mice: 5 days after dysentery infection	4	++	++++	+	+
	Healthy	3	+	+	±	
	Control	3	—	—	—	
III	Spleen of mice: 5 days after dysentery infection	4	++++	++	++++	++++
	Healthy	4	—	—	—	—
	Control	3	—	—	—	
IV	Kidney of mice: 5 days after dysentery infection	4	++++	++++	+	+
	Healthy	4	+	+	+	±
	Control	3	—	—	—	
V	Liver of mice: 5 days after streptococcus infection	4	+	+	+	+
	Healthy	4	+	—	—	+
	Control	3	—	—	—	
VI	Spleen of Mice: 5 days after streptococcus infection	4	++	+++	+++	++
	Healthy	4	—	++	+	+
	Control	3	—	—	—	

Symbols: + scratching nose with paws; ++ the same and sneezing and ruffled fur; +++ the same and coughing, all clearly expressed; ++++ excitement, convulsions, fatal outcome; —absence of these signs.

From Table 2 it is evident that following dysentery and streptococcal infection there are formed universal foreign antigens.

Thus, in the course of a one day and especially a 5-day infection of white mice with dysentery there is formed in their livers, spleens and kidneys foreign antigens (or antigen), but at the same time in their livers a certain quantity of the antigens present are destroyed. In the course of a 5-day streptococcal infection of white mice in the livers and especially in the spleens there is formed foreign antigens and at the same time a certain

TABLE 2

Examination of the Specificity of Foreign Antigens Formed

Experiment series	Sensitizing Material	Number of desensitized guinea pigs	Final reaction to material	
			Original	Heterologous
VII	Spleen of mice 5 days after dysentery infection	2	++ ++	+++ ++
VIII	Spleen of mice 5 days after streptococcus infection	2	+++++++	++ ++

Symbols as in Table 1.

quantity of the antigens present are destroyed. The antigens formed in the spleens of mice during dysentery and streptococcus infection are not specific.

SUMMARY

When applying active anaphylaxis and desensibilization, it was shown that in the case of experimental dysentery infection of white mice, foreign antigens (or antigen) are formed in their liver, and principally in the spleen and kidneys, whereas a certain quantity of the antigens present are simultaneously destructed. The same phenomenon takes place in the liver, and, particularly, in the spleen of mice being experimentally infected with streptococci.

Foreign antigens formed in the liver of mice in the case of the above infections are nonspecific.

LITERATURE CITED

[1] L. A. Zilber, Uspekhi Sovremennoi Biol. Vol. 27, Issue 2, pp. 185-210 (1949).

[2] L. A.Zilber and V. D. Timakov, Zhur. Microbiol., Epidemiol. i Immunobiol* No. 1, pp. 8-13 (1952).

[3] R. V. Petrov and A. I. Ilina, Study of the antigens peculiar to the tissues of irradiated organism.* Theses of the First Soviet Conference on Radiology, 1956.

* In Russian.

EXPERIMENTAL BIOLOGICAL MODEL OF BACILLARY DYSENTERY

V. V. Budylina, A. Yu. Illyutovich, Z. S. Petrova, T. V. Bodulina,
E. E. Golubeva, A. I. Titrova, R. S. Chetvernina

From the Stavropol Research Institute for Vaccines and Sera (Director – V. M. Kruglikov, candidate for Medical Sciences; Scientific Director – V. V. Budylina, candidate for Medical Sciences)

(Received November 26, 1955. Presented by L. A. Zilber, active Member of the Academy of Medical Sciences, USSR)

The basic requirement for the successful pathogenic study of human infectious diseases is the experimental reproduction of the given disease in animals. E. V. Abramova (1938) first demonstrated that the course of bacillary dysentery was similar in monkeys and man. An experimental model of bacillary dysentery was recently perfected on monkeys at the Sukhumsk Medical-Biological Station of the Academy of Medical Sciences USSR, under the direction of V. L. Troitsky. During research on this problem, however, it was found that monkeys in captivity often incur the so-called "spontaneous" Flexner's dysentery, causing attempts to experimentally infect them with the Flexner's type of dysentery pathogens to fail.

We conducted experiments reproducing an experimental model of Flexner's dysentery on kittens. Our choice of this type of animal was based on the research of Grigoryev, who, as early as 1891, demonstrated the possibility of infecting kittens with bacillary dysentery pathogens, and also on the work of Abramova and Burova (Department of Parasitology, VIEM), which established that kittens in captivity incur bloody mucous dysentery, and, therefore, that pathogens of biological properties similar to the Grigoryev-Shiga, Flexner's and Sonnei strains can be obtained from them. By the time the work was completed, we had become acquainted with the data of E. A. Sergeevich (from the Molotov Institute of Vaccines and Sera), which, based on experiments conducted, indicated that the domestic cat could be used as an experimental-biological model for the reproduction of bacillary dysentery.

EXPERIMENTAL METHODS

The experiments were done on 16 kittens ranging in age from 2 to 5 months. The animals weighed from 510 to 1,280 grams. The kittens were previously quarantined and twice examined.

Bacteria of a local strain of Flexner's No. 6176, isolated from a dysentery patient on October 15, 1953, with typical morphological, cultural, biochemical and serological properties and an above average virulence, were used to infect the kittens. The infection was produced by a suspension of microbes in a physiological solution, obtained by washing from a 24-hour agar culture, 1-2-4-8 billion microbic bodies dense, in 1 ml according to the visual standard. The microbe suspension was given to the animals in warm milk after preliminary 24-hour starvation (1 ml of the microbe suspension to 2 ml of milk). The subsequent food ration of the infected kittens consisted of milk, cereal, bread, meat and water. They were fed three times a day.

EXPERIMENTAL RESULTS

During the beginning of the disease, we observed an increased temperature, the appearance of watery stool with pathologic foreign matter, and decline of general condition. In response to the infection, after the

incubation period all 16 kittens presented a clinical picture similar to the clinical picture of dysentery in humans. To confirm the diagnosis of dysentery, we used clinical observation, laboratory examinations, and we studied the pathologico-anatomical picture of the disease and the histo-morphological changes in the organs. In order to observe the development of the disease, the infected animals were sacrificed and autopsied at intervals ranging from the 6th to the 38th day after infection. The average incubation period was 3-6 days; the maximal period was 9 days, the minimal, 2 days.

All the experimental animals were divided into three groups according to the severity of the clinical course of the disease: 1) those with a serious form of dysentery, 2) those with dysentery of average seriousness, 3) those with a light form of the disease.

There were 3 kittens in the first group (No. 1, 8 and 14), one of which (No. 1) died on the 14th day after infection. The clinical picture of the disease in these animals was characterized by a violent beginning (marked increase in temperature, watery, bloody mucous stool, increased frequency of defecation, and marked decline in the animals' general condition—loss of appetite, sluggishness, intoxicated appearance—unsteady gait). Two days before the death of kitten No. 1, vomiting, convulsions and a condition of complete prostration were observed. In all of the kittens, tenesmus, anal distention and weakening of the sphincter were also noted. Bloody mucous material issued constantly from the anal orifice. In this group of animals, we traced the course of the illness up to autopsy (4-6-8 days). For the whole observation period, the kittens retained the increased temperature and pathologic stool, as well as the poor general condition.

The second group consisted of 5 kittens (No. 9, 10, 11, 13 and 16). These animals had a typical dysenteric affection, and their general condition was considerably disturbed. In all of the kittens, we observed a violent beginning period with a sharp increase in temperature, the appearance of frequent watery stool with pathologic foreign matter; in 3 kittens, blood and mucous were observed in the stool. The development of the disease was attended by tenesmus, anal distention, and weakening of the sphincter with involuntary defecation. These symptoms developed on a background of significant disturbance to the animal's general condition. The kittens lost their appetite and became sluggish as early as 1-2 days before becoming ill. On the 5th day of the disease, kitten No. 13 manifested an unsteady gait and convulsions.

The acute period of the illness lasted 3-6 days, and the high temperature continued for approximately the same period. In the kittens with a longer observation period (No. 11 and 16), the temperature fell below normal on the 6th-7th days of the disease and remained so until the end of observation. The watery stool remained during the entire observation period; blood was recorded in the stool during the 1st-4th days, mucous during the 2nd-9th days of the illness.

There were 8 kittens in the third group (those with a light form of dysentery). They all had a typical dysenteric affection without the symptoms of intoxication and with a relatively satisfactory general condition. As a rule, the majority of them increased in weight during the observation period.

In this group, the disease began with a slight or even normal temperature. In three of the kittens, the temperature only rose on the 5th-7th day of the disease. Frequent, watery stool appeared on the first day of sickness. Mucous was discovered in the stool of 5 kittens (see table). The kittens of this group were observed for a comparatively long period (up to 38 days), and it could be established that formed stools appeared on the 12th-16th days of the disease, although watery stools occurred periodically. The increased temperature was retained for 3-16 days, after which days of normal temperature alternated with slight exacerbations. One can also note that the longest incubation periods were most frequently recorded in the kittens of this group.

The clinical picture of the disease in all these three groups of animals indicated, first, that the disease appeared after an incubation period, second, that it was characterized by symptoms typical of dysenteric disease in man (especially in children), and third, that a definite pattern could be established in the development of the infection (acute period, followed by the gradual disappearance of the disease).

The development of clinical forms of the disease which varied as to gravity demonstrates the individual reactivity of the animals to the introduction of disease pathogens into the body.

We analyzed the possibility that the severity of the disease depends on the number of microbes which the animals received, or on the weight of the animal. We found that, in a given case, there was no relation between these factors. Thus, kitten No. 1, which had received 1 billion microbic bodies in 1 ml, had the serious form

of the disease, ending in death, while kitten No. 4, which had received 8 billions of microbes per 1 ml, was sick from a light form of dysentery. Both animals weighed approximately the same (kitten No. 1 – 510 g, kitten No. 4 – 640 g).

To confirm the clinical diagnosis of dysentery in the kittens, we conducted laboratory bacteriological examinations on the feces of the sick animals. The analyses were done daily from the 2nd day after infection until the end of observation. Bacteriological confirmation was received in all 16 kittens. Positive bacteriological cultures were recorded during the first 7 days of sickness. According to the frequency with which dysenteric bacilli were discovered, positive cultures were recorded $2\frac{1}{2}$ times more often in the first two groups (8 kittens) than in the last group (also 8 kittens), in spite of the fact that the observation period was longer in the last group. The more frequent cultures obtained of dysentery bacilli in the first two groups depended directly on the character of the stool, since the watery stools with blood and mucous gave 88% positive cultures, the watery stools with mucous, a little more than 50%, the watery stool without foreign matter, 26%, and the formed stool, a total of 2.4% positive cultures.

Bacteriological examinations were done on the internal organs of 15 of the animals after autopsy. For the examination, we used: blood from the heart, urine, bile, sections of the liver, spleen, mesenteric lymph nodes, kidneys and bone marrow, and also the contents and scrapings from the mucosa of the large and small intestines. The material was immediately cultured on favorable media. The dysenteric bacilli were isolated by the usual method.

Bacilli of Flexner's dysentery, type "W", were cultured from 15 kittens. Complete (100%) positive cultures were obtained from the contents of the rectum and from the mesenteric lymph nodes. In 13 (out of 15) kittens, pathogens were cultured from the spleen, in 11, from the contents and scrapings from the mucosa of the small intestine, in 7, from the contents and mucosa scrapings of the transverse colon, and in 5, from scrapings of the mucosa and the contents of the ascending colon. Dysentery bacilli were cultured from bone marrow in three kittens, and from the kidneys, in two.

To determine the immunobiological response of the body to the infection, we set up a reaction with the blood serum of the sick animals to obtain the level of agglutinin antibodies in the blood, and also specific and nonspecific phagocytic reactions to evaluate the change in cell reactivity.

The observations were made on 10 kittens. The production rate of agglutinin antibodies was determined in dynamics. The reaction was set up 3 to 6 times. Thirty-nine reactions in all were conducted. Each reaction was done with a live 18-hour culture of Flexner's type "W" No. 6176 in a dilution of $\frac{1}{3200}$ (see table).

The Dynamics of Agglutinin Production in the Blood Serum of the Infected Animals

No. of experiment	No. of kitten	Observation period (in days)	Number of reactions set up	Day after infection on which reaction was set up					
				3rd day	5th day	9th day	13th day	16th day	21st day
1	7	6	2	1/50	1/200	·	·	·	·
2	8	10	3	—	1/200	1/200	·	·	·
3	9	12	4	—	1/100	1/100	1/400	·	·
4	10	15	5	—	1/100	1/100	1/400	1/400	·
5	11	20	5	—	1/200	1/200	1/200	1/400	·
6	12	25	6	1/50	1/200	1/400	1/400	1/400	1/400
7	13	7	2	—	1/100	·	·	·	·
8	14	12	3	—	1/100	1/100	·	·	·
9	15	14	4	—	1/400	1/400	1/400	1/200	·
10	16		4	—	1/400	1/400	1/400	1/200	·

Explanation of symbols used: – ; negative reaction; · ; reaction not set up.

In analyzing the results obtained, one can note that a positive reaction appeared on the 6th day after infection, i.e., on the 1st-4th day of the sickness. On the 9th to 13th days after infection, the antibody titer

continued to increase (kittens No. 9, 10 and 11). In kitten No. 12, on the 15th day of sickness (the 21st day after infection) no decrease in the antibody titer could as yet be observed. The dynamics of the reactions in our experiment certainly reconfirm the development of a specific infection process in the animals. As for the comparatively slight elevation of the antibody titer (positive reaction in a dilution of $^1/_{400}$), analogous features have been noticed, as we know, in the results of the Widal test for dysentery in humans.

To further characterize the immunobiological response in the bodies of the infected animals, we conducted specific and nonspecific phagocytic reactions. To set up the reaction in the kittens, we took blood from the auricular vein in the morning on an empty stomach and mixed it with a 2.5% solution of sodium citrate in the following proportion: one part of citrate to one part of blood. To the blood citrate, we added one volume of an emulsion of a one-day culture of microbes, which had 1 billion microbes according to the visual standard. The mixture was put in an incubator for 30 minutes, after which thin smears were prepared. For the specific reaction, we used a strain of Flexner's "W" No. 6176 as the test microbes, and for the nonspecific, a culture of intestinal bacilli. As the activity index of the phagocytic reaction, a quantity of phagocytizing neutrophils, expressed in percentages was used. The intensity of phagocytosis was computed from the amount of bacilli in one microphage (arithmetic mean number). Observation was done on 10 kittens. We set up a series of reactions (on the 5th, 10th, 15th and 18th days after infection, and also before infection) in order to evaluate the phagocytic reaction in dynamics at different stages in the development of the infection. Even before infection, the indices of leukocyte activity were considerably lower in the specific reaction than in the nonspecific, which indicates the more active phagocytosis of the common pathogenic flora (the intestinal bacilli in particular).

When the dynamics of the phagocytic reaction was compared with the severity of the clinical picture of the disease in the first and second groups of animals (those with the more serious form of the disease), the indices of the activity and intensity of the leukocytes generally tended to decline sharply during the whole observation period both in the specific and in the nonspecific phagocytic reactions, which indicates the inhibition of both the specific and general reactivity of the body in the animals affected by dysentery.

The restoration of body reactivity proceeded more rapidly in the third group with the light form of dysentery, according to the indices of activity and intensity of the leukocytes to phagocytosis, and the index of phagocytosis intensity after the 10th post-infection day (4th-6th day of sickness) exceeded the original indices before infection (1.3-2.4).

According to the indices of accumulation in the blood of agglutinins and of change in cell reactivity, the immunobiological response of the body confirmed the development of a specific infection in the animals.

The dysentery diagnosis was also confirmed by the pathologico-anatomical picture of the disease. During the acute period of the disease, autopsy determined the following in the large intestine: hyperemia and edema of the mucosa, muco-purulent material, and punctate hemorrhages; in the small intestine: hyperemia, edema of the mucosa, and a small amount of muco-purulent material. During the second period of the disease (9th-18th days of sickness), the mucosa of the large and small intestines was pale, atrophic and its contour frequently blurred (especially in the small intestine). In the other internal organs, the following were observed during the acute period of the disease: the mesenteric lymph nodes were enlarged and dense; the liver was plethoric and bloody fluid oozed from a cut section; the spleen was more frequently enlarged and purplish-blue in color, with an uneven, granular surface. During the second half of the disease, areas of a clay-like color were observed in the liver, and the hepatic tissue was flaccid. The spleen remained enlarged and edematous and a sanious fluid oozed from a cut section; the kidney pattern was more frequently blurred.

The microscopic picture of the changes in the internal organs was studied in 12 kittens.

In the large intestine during the acute period of the disease were observed: desquamation of the surface epithelium, destruction of the connective tissue with the formation of microscopic ulcers, edema of the submucosa, changes in the vascular walls (especially in the submucosa) and the formation of a film on the mucosa with varying degrees of contact with the underlying tissue.

Along with degenerative processes, in the large intestine, by the 11th-12th day after infection, one could observe the regeneration of the mucous membrane, which was expressed in the growth of connective tissue replacing the destroyed mucosa. We did not observe the regeneration process to be completed even by the 25th day after infection.

The main microscopic observation in the mucosa of the small intestine was the destruction of its villi—considerable destruction to the actual membrane of the villi was observed as well as to their epithelium. This process began as early as the 6th day after infection, and reached its highest intensity by the 12th day. Other observations were: the infiltration of the mucous membrane, edema of the submucosa, dilatation of the mucosal vessels and deposits on the mucosal surface which were smaller than in the large intestine. Regeneration processes were observed in the small intestine as early as the 7th day after infection, although the regeneration of the mucous membrane had still not been completed by the 25th day after infection.

Microscopic observations in the liver were: dilatation of the portal vein capillaries and vacuolization of the hepatic cells, which was greater on the periphery of the lobule than in its center. In the red pulp of the spleen, an afflux of blood and a dark brown pigment were observed. In the same organ, the centers of multiplication of the white pulp were obscured, which could also be observed in the lymph nodes. In the cardiac muscle (especially during the second half of the disease), we found small foci of inflammation and the disintegration of the muscle fibers. In the kidneys, dilatation of the vessels could be seen, particularly on the boundary between the cortical and medullary substances. Some vacuolization of the tubular cells was also noted.

To conclude, one must remark that the profundity and character of the morphological changes do not depend on the severity of the disease; the time at which the animals were autopsied after the infection is the important thing here.

From the clinical and laboratory observations we made on the infected animals, we consider it possible to conclude that, in the conditions of the experiment, kittens manifest a typical dysentery and may serve as an experimental model for studying various problems of pathogenesis and immunity in dysentery.

SUMMARY

Experiments were carried out aimed to reproduce the experimental model of Flexner's dysentery in kittens.

All the kittens infected with the typical Flexner's strain showed after the incubation period (5 to 6 days) clinical symptoms similar to that of the dysentery in man.

Clinical and laboratory observations on the infected animals make it possible to assume that the kittens may serve as a model for studying problems of pathogenesis and immunity in bacillary dysentery.

EXPERIMENTAL BIOLOGY

CELL DIVISION AND VITAMIN A

I. A. Alov

From the Dept. of Histology (Chief Assistant – Prof. I. A. Alov), Khabarovsk Medical Institute

(Received June 20, 1956. Presented by Prof. D. N. Nasonov, Active Member of the Academy of Medical Sciences, USSR)

Cell multiplication in the different tissues is controlled by neurohumoral regulation, which is accomplished both by regulating mechanisms (the nervous system, the hormones of the suprarenal, hypophisis, thyroid and sex glands), and by certain local factors (cell functional activity, the process of cell differentiation, tissue alteration, etc.). The mitotic rate of the tissues evidently also depends on the exchange of the vitamins which play an important part in the different physiological and biological processes. Certain observations [7, 9] permit the hypothesis that vitamin A is one of the essential links of the cell division regulation system.

The purpose of this work was to study the role played by vitamin A in regulating cell division.

EXPERIMENTAL METHODS AND RESULTS

We conducted three sets of experiments. In the first set, the influence of vitamin A injections on the intensity of cell division in different organs of white mice was studied.

A butyrous solution of vitamin A was injected subcutaneously in 500, 1000, or 3000 nternational nits per 24 hours. The injection was repeated after a period of 5 days (the last injection being 7 hours before death). The control group of mice was injected with an equal quantity of butter. All the animals were kept in identical conditions and on a regular diet. Mitotic activity was studied in the epithelium of the cornea, intestine and tongue. The intensity of the mitoses was judged according to the number of cells dividing in a constant area (1.65 mm^2) and according to the coefficient of the phases (the relation of the first two phases of mitosis to the last two phases). Calculations were done on total preparations of the cornea and sections (10 microns) of the cornea and other organs.

The results of the experiments are given in Table 1.

TABLE 1
The Influence of Vitamin A on Cell Division

Mouse group	Number of animals	Intensity of mitoses (M ± m) and coef. of the phases (K)		
		Cornea	Intestine	Tongue
Control	7	105± 6.7; K=2.4	273±10; K=1.2	27±0.8; K=1.8
Vitamin A, 500 i.u.	8	101±12.7; K=2.2	368±10; K=1.5	29±0.7; K=1.7
Control	6	115±18; K=1.4	289± 2.5; K=1.3	22±0.6; K=2.0
Vitamin A, 1000 i.u.	6	172±12; K=1.2	640±15; K=1.3	36±3.0; K=2.2
Control	9	59± 8.8; K=1.9	391± 7.0; K=1.6	23±5.2; K=2.8
Vitamin A, 3000 i.u.	9	111±15; K=1.7	337±17; K=1.7	48±5.0, K=2.7

As is evident from Table 1, vitamin A stimulated cell division. When relatively small quantities of the vitamin were injected (500 i.u.), mitotic intensity increased in the intestinal epithelium only. Larger doses of the vitamin (1000 i.u.) caused mitotic stimulation in the spithelium of the cornea and of the tongue. In one of the experimental groups (data not included in Table 1), the number of dividing cells in the epithelium of the tongue did not increase, and growth was noted only in the early phases of mitosis (increase of the coefficient), which seemed to be associated with change in the multiplication rate.

That the degree of increase in mitotic activity varied in the different groups of animals was probably due to their individual singularities and to the pre-experimental diet regime. The most significant mitotic stimulation was found in the intestinal epithelium. The intestinal epithelium also reacted to hypervitaminosis earlier than the others by suppressing mitotic activity. The 30001.u. injection of vitamin A stimulated mitotic activity in the epithelium of the cornea and tongue, but, in the majority of mice, lessened it in the intestinal epithelium. Since increased mitoses were noted in the intestinal epithelium of some mice, the average indices of mitotic activity with the 300(i.u. vitamin A injection, given in Table 1, were slightly lowered. The high sensitivity of the intestinal epithelium to vitamin A seems to be explained by the fact that this organ, as does the liver, forms vitamin A from carotene [8]. In the second series of experiments, changes in mitotic rate under conditions of vitamin A deficiency were studied.

White mice were put on a vitamin A deficient diet for 20 days: wheat flour, lard, yeast and a salt mixture [5]. The control animals were kept on their regular diet.

By about the 20th day, a typical picture of vitamin deficiency developed in the experimental mice (growth sharply arrested, loss of weight, dishevelled fur). Avitaminosis did not result in the development of xerophthalmia in any of the experiments.

The results of the experiments are given in Table 2.

TABLE 2
Mitotic Activity Change in A Avitaminosis

Mouse group	Number of animals	Intensity of mitoses (M ± m) and coefficient of the phases (K)		
		Cornea	Intestine	Tongue
Control	9	97±13.0; K=1.7	347±18; K=1.6	27±3.4; K=2.0
Avitaminosis	7	91±13.0; K=1.6	336±20; K=1.6	27±5.3; K=2.5
Avitaminosis + thyroidin	7	161±15.0; K=1.8	299±16; K=1.5	33±6.0; K=3.6

As Table 2 shows, vitamin A deficiency in the experimental animals did not cause any appreciable change in mitotic activity. Only when the preparations were stained on to ribonucleic acid according to Brash could one note certain histochemical changes in the mitotic process of the intestinal epithelium: the normal gradual decrease in the concentration of ribonucleic acid could not be observed in part of the dividing cells, and the high ribonucleic acid content was retained in the metaphase.

When we analyzed the results of both series of experiments, we noticed that the influence of vitamin A on the mitotic process of the tissues was very similar to that of the thyroid gland. Thyroidin stimulates mitotic activity just as vitamin A does. Thyroidin had a greater activating influence on cell division, but it, too, was most sharply expressed in the intestinal epithelium. Methylthiouracil blockade of the thyroid gland in adult animals, like A avitaminosis, did not cause any appreciable change in mitotic activity [1].

These comparisons led us to include a third group of animals in the experiments with avitaminosis, which were given thyroidin with their food (18 mg of thyroidin per mouse in a period of ten days) on a background of vitamin deficiency. In this group of mice (Table 2), as is usual in experiments with thyroidin, a rather considerable

increase in mitotic activity was observed in the corneal epithelium. Only a small increase was observed during the early mitotic phases in the epithelium of the tongue. In the epithelium of the intestine (the organ most sensitive to thyroidin and vitamin A), mitotic stimulation not only did not occur, but mitotic activity was even somewhat decreased.

Thus, although avitaminosis was not attended by noticeable change in the number of dividing cells, it did lead to profound disturbance in the regulation of cell division. In vitamin A deficiency conditions, a powerful mitosis stimulator – thyroidin – did not cause an increase in mitotic intensity in the epithelium of the intestine or the tongue. Since the thyroidin hormone is the activator of carotenase (the enzyme which transforms carotene into vitamin A), one can propose that the thyroid gland acts on cell division through the synthesis of vitamin A. In every case, the thyroid gland hormone could only regulate the mitoses if vitamin A was present in the tissues. There are evidently similar correlations between other hormones that regulate cell division and vitamins. Adrenalin, in particular, seems to belong in this group as its antimitotic action was not discernable in guinea pigs deprived of the ability to synthesize ascorbic acid [6].

We have demonstrated in previous papers [1, 2, 3, 4] that the different factors of neurohumoral regulation of cell division in normal tissues (adrenalin, cortisone, thyroidin) do not change mitotic activity in tumors.

In a third series of experiments, we studied the influence of vitamin A on cell division in mice with Kroker's sarcoma. Beginning the 9th day after the sarcoma graft, the mice were injected with 1000 i.u. per day of vitamin A for 5 days.

TABLE 3

Vitamin A Influence on Cell Division of Mice with Grafted Tumors

Mouse group	Number of animals	Intensity of mitoses (M ± m) and coefficient of phases (K)		
		Cornea	Intestine	Sarcoma
Control	8	123±16; $K=1.8$	331±16; $K=1.3$	179±8.3; $K=2.6$
Vitamin A, 1000 i.u.	9	138±16; $K=1.2$	443±18; $K=1.7$	178±6.3; $K=2.7$

The results of the experiments (Table 3) showed that the vitamin A injection stimulated mitotic activity in the intestinal epithelium of the mice with the sarcomas. Mitotic intensity did not change in the corneal epithelium of these animals. Evidently, the reaction of normal cells to the action of vitamin A is lessened under the influence of the growing tumor. However, it would be premature to draw final conclusions as the reaction of the tissues to the vitamin injection varied considerably as to intensity in the different groups of mice (Table 1).

In the actual tumor, mitotic activity was not affected by vitamin A. Mitotic intensity in the tumors of both the experimental and control mice remained the same. Thus, vitamin A, like adrenalin, cortisone and thyroidin, does not influence the division of tumor cells. Cell division in tumors is out of the control of the system regulating these processes in normal tissues.

At this time, it is still difficult to explain the relative areactivity of the tumor cells. If one agrees that cell division in normal tissues is connected with nucleic exchange, then the different mitotic regulators, all things considered, seem to effect mitotic activity through cell nucleic exchange. Perhaps other exchange processes cause division in tumor cells. It is also possible that the nucleic exchange of tumor cells is different, and that the mitotic regulators do not change it, or even that, in a tumor cell, those intermediate links through which the mitotic regulators change the nucleoprotein exchange drop out. The first proposal is less probable: the experiments conducted in our L. M. Ermolenko laboratory showed that blockading nucleic exchange by different inhibitors deters cell division in both normal and tumor tissues. Changes in the actual nucleic exchange of the tumor cells or those intermediate links through which the mitotic regulators influence nucleic exchange are more probable. The possibility of such changes is indicated by the second series of experiments, in which cell reaction to thyroidin was disturbed by the exclusion of vitamin A from the exchange. Nor must one exclude the possibility

that similar correlations are formed in the growing tumor. Further experiments are needed to prove the validity of this working hypothesis.

SUMMARY

Vitamin A injected into white mice stimulates cell division. The epithelium of the intestines is the most sensitive to the vitamin. Vitamin A deficiency does not lead to any considerable changes in the number of mitoses in the tissues but the regulation of cell division is strongly disturbed. In cases of avitaminosis even such a powerful stimulator of mitosis as thyroidin fails to increase mitotic activity. The presence of vitamin A in the tissues is indispensable for the stimulative effect of thyroidin on cell division. Experiments on mice with Kroker's sarkoma demonstrated that vitamin A does not change the intensity of tumor cell division, since in tumors that process is out of control of the neurohumoral system which regulates the mitotic activity in normal tissues.

LITERATURE CITED

[1] I. A. Alov, Byull. Eksptl. Biol. i Med. Vol. 40, No. 12, pp. 47-50.

[2] I. A. Alov, Voprosy Onkol. 1955, Vol. 1, No. 6, pp. 40-45.

[3] I. A. Alov, Doklady Akad. Nauk SSSR 1956, Vol. 107, No. 5, pp. 745-747.

[4] I. A. Alov, in the book: Works of the Khabarovsk Medical Institute,* 1956, Vol. 15, pp. 63-71.

[5] P. P. Smilyanskaya and K. Z. Tulchinskaya, in the book: Vitamins in Theory and Practice,* Moscow 1953, pp. 152-153.

[6] L. V. Suvorova, Reactive Inhibition of Cell Division in Animals and the Development of a Capacity for it in Ontogenesis,* Dissertation, Leningrad 1955.

[7] E. Bujard, W. Jadassohn and R. Brun, Compt. rend. soc. biol.* 1954, Vol. 148, No. 9-10, pp. 778-780.

[8] R. F. Krause and H. B. Pierce, Arch. Biochem. 1948, Vol. 19, pp. 145-148.

[9] A. Studer and I. A. Frey, Dermatologica, 1952, Bd. 104, N. 1, S. 1-18.

* In Russian.

THE ROLE OF THE THYROID GLAND IN THE REGULATION OF CELL DIVISION

I. E. Epova

Dept. of Histology (Chief – Assistant Prof. I. A. Alov), Khabarovsk Medical Institute

(Received July 17, 1956. Presented by D. N. Nasonov, Active Member of the Academy of Medical Sciences, USSR)

Cell division in the various tissues is controlled by the neurohumoral regulator system of the body. The thyroid gland plays an important part in this regulating system. I. A. Alov's experiments [1] with thyroid hormone injections and methylthiouracil blockading of the thyroid gland have shown that the thyroid hormone is a powerful stimulator of cell division. Other authors, on the basis of almost identical experiments, are inclined to consider the thyroid hormone as an antimitotic agent [6, 7, 8].

The purpose of this work was to clarify the importance of the thyroid gland in the regulation of cell division, by using other methods to stimulate and suppress its function.

EXPERIMENTAL METHODS AND RESULTS

In the first series of experiments, we studied mitotic activity change in various organs when the thyroid gland had been completely removed.

The experiments were done on white rats. The thyroid gland was removed bilaterally by the usual method. After the death of the animals, histological inspection of the operation region checked the completeness of the gland's extirpation. Mitotic activity was determined only in those animals in which the gland had been completely removed. In the control animals, the thyroid gland was exposed by operation, but not damaged or removed. The experimental and control animals were killed 10 hours after the operation, and mitotic intensity was determined in the epithelium of the cornea, intestine, tongue and skin. Mitotic intensity in the tissues was judged according to the amount of dividing cells in an area of 1.65 mm^2 and according to the coefficient of the phases (the relation of the two first mitotic phases to the two ensuing ones). The dividing cells were counted on four sections (10 microns) taken from one region, 100 microns apart. Mitotic activity in the cornea was determined on total preparations. The data was statistically processed by the Fisher-Styudent method.

Table 1 shows the results of the first series of experiments.

TABLE 1

Change in Mitotic Intensity After Extirpation of the Thyroid Gland

Animal group	No. of animals	Number of mitoses (M ± m) and coefficient of the phases (K)			
		Cornea	Intestine	Tongue	Skin
Control	6	99 ±6.5 K=3.2	399±15.0 K=4.2	40.3±4.5 K=2.4	36±1.5 K=4.3
Thyroid gland extirpation	7	60.0±6.9 K=3.6	315± 5.4 K=4.3	34.6±5.3 K=3.0	26±1.5 K=3.9

Thus, complete removal of the thyroid gland caused mitotic activity to decrease. Ten days after the extirpation of the gland, mitotic intensity had decreased $1\frac{1}{2}$ times in the epithelium of the cornea, intestine, tongue, and skin. This same relatively small decline in mitotic activity was also observed in experiments with 6-methylthiouracil blockade of the thyroid gland [1, 2]. Evidently, the other mechanisms regulating cell division can partly compensate for the exclusion of the thyroid gland.

In a second series of experiments, we studied mitotic activity change in the same organs with a hyperfunctioning thyroid gland.

White mice were injected with a thyrotropic hormone. To prepare the thyrotropic hormone, acetonizing powder was extracted from the anterior lobe of the hypophisis of large horned cattle by a 0.1 N solution of $NaHCO_3$ for a period of 24 hours in the cold. For a period of 8 days, the mice were injected subcutaneously with 0.3 ml of the extract twice daily (a dose equivalent to 10 ml of the acetonizing powder of the hypophisis per 100 g of weight). The control animals were injected with a corresponding amount of a physiological solution. After the death of the mice, the thyroid gland was examined histologically.

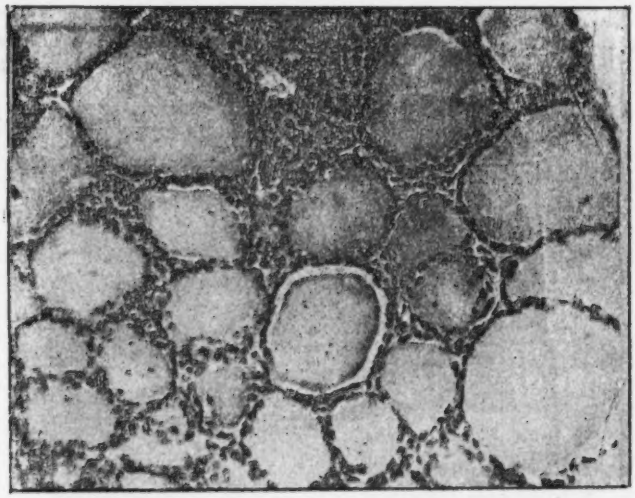


Fig. 1. White mouse thyroid gland under conditions of normal maintenance of the animal.

A repeat injection of the thyrotropic hormone given the white mice caused activation of the thyroid gland. The gland increased in size and incurred hyperemia; the follicular epithelium became cylindrical in form; the central follicles grew smaller and partially emptied; the colloid became vacuolized, and epithelial proliferation was observed (Fig. 1, 2). At the same time that the functional activity of the thyroid gland increased, change of mitotic activity occurred in the experimental organs (Table 2).

Mitotic intensity increased $2\frac{1}{2}$ times in the epithelium of the cornea and tongue, $1\frac{1}{2}$ times in the epithelium of the intestine, and $2\text{-}2\frac{1}{2}$ times in individual experiments.

The results of the experiments show that the thyrotropic hormone is a stimulator of cell division in the body. Thyrotropic hormone activation of the thyroid gland caused mitotic activity in the different organs to appreciably increase. The removal of the thyroid gland is attended by a decrease in mitotic activity.

Several authors [3, 4, 5] have shown that one of the activators of the thyroid gland is the process of daily illumination. Accessory illumination of the animals also caused mitotic activity to increase [6]. Comparing these facts, we decided that the mitotic stimulation effected by light is probably realized through the thyroid

TABLE 2

Change in Mitotic Intensity When the Thyroid Gland is Activated by the Thyrotropic Hormone

Animal group	Number of animals	Number of mitoses (M ± m) and coefficient of the phase (K)		
		Cornea	Intestine	Tongue
Control	8	92.3±7.9; $K=1.0$	324±15; $K=1.5$	23±1.7; $K=1.6$
Thyrotropic hormone	15	192.8±8.5; $K=1.1$	491±23.9; $K=1.0$	63.8±3.8; $K=1.0$

gland. We conducted a third series of experiments to study the role of the thyroid gland in the activation of cell division under conditions of accessory illumination of the animals.

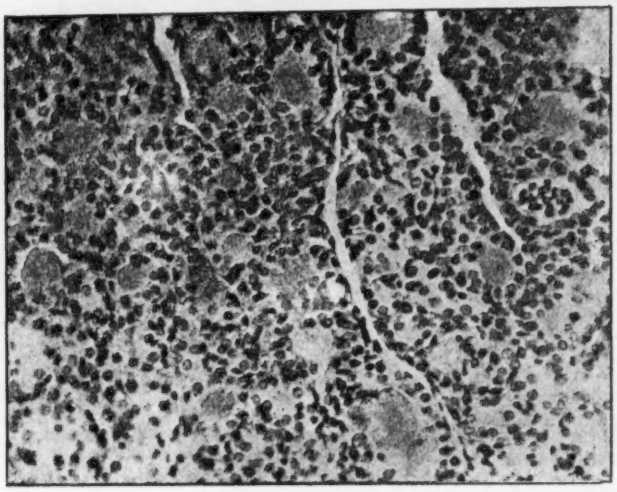


Fig. 2. Thyroid gland of a white mouse after the thyrotropic hormone injection.

The first group of experiments in this series studied mitotic activity change under conditions of accessory illumination. The experiments were conducted in March and April on white mice. The experimental animals were kept in glass jars and given accessory illumination daily for 10 days for 5-6 hours, beginning at 7 p.m. (electric 60 w bulb at a distance of 2 meters). The control group of mice were kept in cells, without accessory illumination.

The results of the experiments are given in Table 3.

As is evident from Table 3, with accessory illumination mitotic intensity increased $2^1/_2$ times in the epithelium of the cornea; in the epithelium of the tongue, there was no essential change. Changes in the structure of the thyroid gland attesting to its increased activity were simultaneously observed. In the thyroid glands of the majority of mice, increase in the height of the follicular epithelium, vacuolization of the colloid, and, in some experiments, the almost complete disappearance of the colloid were observed (Fig. 3). The degree of change in the thyroid gland varied in different animals; in all cases it was considerably lower than when the gland was activated with the thyrotropic hormone.

TABLE 3

Change in Mitotic Intensity due to Accessory Illumination

Animal group	Number of animals	Number of mitoses (M ± m) and coefficient of the phases (K)		
		Cornea	Intestine	Tongue
Control	13	60.9± 9.7; $K=1.1$	291±15.5; $K=0.9$	46± 8.1; $K=1.0$
Illumination	13	161±13.3; $K=1.2$	428±19.2; $K=0.8$	42± 7.3; $K=1.4$
Control + 6-methyl-thiouracil	18	95.2±13.8; $K=1.3$	302.5±16.9; $K=1.0$	18.9±14.5; $K=1.0$
Illumination + methyl-thiouracil	9	85±11; $K=0.9$	278±11.8; $K=0.9$	15± 3.2; $K=1.1$

These observations allow the hypothesis that mitotic activity change in accessory illumination is associated with the activation of the thyroid gland by light. To verify this hypothesis, a second group of experiments was conducted in this series.

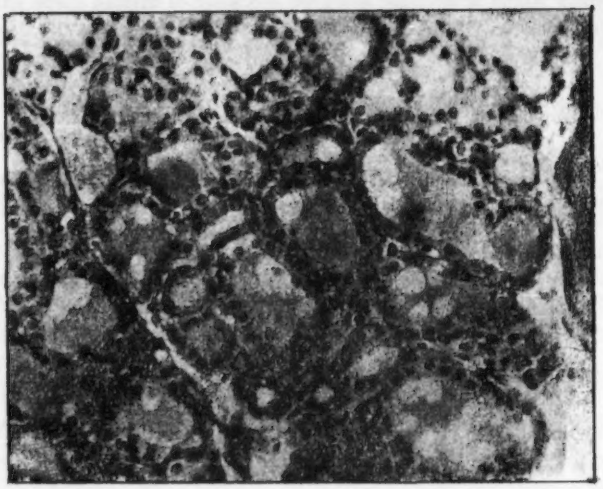


Fig. 3. Thyroid gland of a white mouse after accessory illumination.

The conditions of maintenance and accessory illumination for the experimental and control animals were the same as in the first group of experiments. Five days before the beginning of the experiment, we began to add 6-methylthiouracil, which causes hypofunction of the thyroid gland, to the food of the experimental and control animals (5 mg a day per animal). Methylthiouracil administration was continued during the accessory illumination period, and, in this way, the experimental and control animals received this preparation for a period of 15 days.

The 6-methylthiouracil administration caused a typical goiter-forming reaction (hypertrophy of the gland, increase in epithelial height, proliferation, emptying of separate follicles).

Mitotic activity change in the epithelium of the cornea, intestine and tongue showed that, when the thyroid

gland was blockaded by 6-methylthiouracil, accessory illumination of the animals did not stimulate mitotic activity (Table 3). Moreover, in those mice which had been subjected to accessory illumination, there was even a slight (statistically unreliable) decrease in the intensity of cell division. Therefore, one must also note that the mice which had received 6-methylthiouracil did not endure accessory illumination as well as the normal animals in the first group of the experiments.

The results of this experimental series imply that the increase in mitotic activity which occurs in accessory illumination is accomplished through the thyroid gland. Activation by light of the thyroid gland causes the intensity of cell division to increase, as do the other methods of intensifying the gland's function.

Thus, the experiments described show that the thyroid gland plays an important role in the regulation of cell division in the body.

The thyrotropic hormone is a powerful stimulator of cell division, and the activation of cell multiplication by different environmental factors (in our experiments, light) is, in a series of cases, done through the thyroid gland.

SUMMARY

Experiments carried out on white rats have shown that the complete extirpation of the thyroid gland decreases mitotic activity in different tissues. Injection of thyrotropic hormone increased thyroid gland and mitotic activity. The latter is also increased in the case of accessory illumination of animals. This reaction proceeds by means of the thyroid gland activation; after the blockade of thyroid gland with methylthiouracil, accessory illumination of mice does not bring about an increase of cell division in the tissues.

LITERATURE CITED

[1] I. A. Alov, Byull. Eksptl. Biol. i Med, 1955, Vol. 40, No. 12, pp. 47-50.

[2] A. A. Voitkevich, Bull. acad. sci. USSR, Ser. biol., 1945, No. 4, pp. 385-395.

[3] A. A. Voitkevich, Fiziol. Zhur. SSSR, 1948, Vol. 34, No. 6, p. 722.

[4] B. I. Noyikov and L. Favorova, Doklady Akad. Nauk SSSR, 1947, Vol. 58, No. 4, pp. 701-704.

[5] I. A. Utkin, Byull. Eksptl. Biol. i Med. 1953, Vol. 36, No. 7, issue 1, pp. 36-40.

[6] G. K. Smelser, Anat. Rec., 1947, Vol. 97, No. 3, pp. 369-370.

[7] G. K. Smelser and V. Ozanis, J. Cellular Comp. Physiol., 1954, Vol. 43, No. 1, pp. 107-117.

[8] K. Stein, S. Quinby, and A. Moellen, Anat. Rec., 1947, Vol. 99, pp. 249-264.

ON THE CAUSES OF REGENERATION ABILITY LOSS IN THE AXOLOTL EXTREMITY AFTER X-RAYING

V. F. Sidorova

From the Laboratory of Growth and Development (Chief – Prof. M. A. Vorontsova),*
Institute of Experimental Biology (Director – Prof. I. N. Maisky),
Academy of Medical Sciences, USSR, Moscow

(Received May 8, 1956. Presented by N. N. Zhukov-Verezhnikov,
Active Member of the Academy of Medical Sciences, USSR)

That the axolotl or triton extremity loses its ability to regenerate after exposure to large doses of roentgen rays is known. After the irradiated organ has been amputated, the wounded surface of the skin closes, and the morphogenetic process normally present, which leads to the rebuilding of the lost structures of the organ, is gone. Biological interpretation of the processes occurring in the irradiated organ has been recently done by G. S. Strelin and his colleagues [5, 6], and also by other authors.

However, in none of these hypotheses as to how the regeneration ability is lost by irradiated tissues is there any clear explanation of the reasons for this phenomenon [1, 8, 9, 11].

Such a situation is explained by the difficulties of studying the irradiated organ.

With the doses of radiation customarily used to suppress the regeneration ability of the axolotl or triton extremity (5,000-10,000 r), the morphological picture of the reaction to irradiation is rather indefinite. In some cases, the reaction was observed from the standpoint of the cutaneous sheath: due to the partial destruction of pigment, the skin became a whitish shade. Sometimes the reaction was observed from the standpoint of the digits: resorption of their tips occurred. Essential morphological changes could not be found by the usual methods of study (fixation by Bouin's fluid, staining according to Van Giesen, Mallory, or with hematoxylin-eosin) even in the internal tissues of the extremity.

Thus, histological study of the irradiated limb gives no reason to connect the loss of the regeneration ability with any of the visible morphological changes.

One must note that the sensory and motor reactions were completely retained after irradiation. One can only judge whether the irradiated extremity has lost its regeneration ability after the latent period (3-4 weeks) and subsequent amputation.

The absence of important morphological changes in the tissues of the irradiated extremity, the preservation of the sensory and motor reactions, the presence of a latent period – all these complicate the explanation of the reasons for the loss of the regenerative ability.

In our study, we decided to analyze the role of the nervous system in the process of tissue loss of regenerative ability after irradiation.

There are reports in the literature that, normally, the nervous system plays an important role in the differentiation of the regenerated axolotl limb and in the sequence of formation of the organs's structures [4, 7, 10, 12, 13]. There is also data to indicate that in a denervated axolotl extremity, which has been irradiated by roentgen rays, restoration of the motor and sensory functions occurs more slowly than in nonirradiated extremities [2].

* Deceased.

Retarded restoration of function in the denervated axolotl extremity was observed not only when the actual extremity was irradiated, but when the portion of the spinal cord from which the nerves innervating the extremity originate was irradiated. The same irradiation of another portion of the spinal cord (for example, the lumbar section, when an anterior extremity has been denervated) did not retard functional restoration of the extremity [3].

To explain to what extent the loss of the regenerative ability in the irradiated portion is connected with changes in the nervous system, one must examine the condition of all parts of the nerve course. The changes caused by irradiation can affect the structural features of the peripheral nerve endings or can even impinge on the central nervous system.

EXPERIMENTAL METHODS AND RESULTS

To examine the condition of the nerve endings in the irradiated organ, we used the stump of an axolotl extremity which did not regenerate after amputation. The irradiated extremity (radiation dose, 5,000 r) was amputated a month after the action of the radiation dose. After 4 months, the amputated extremity showed no signs of regeneration. Only at the most distal end of the stump was a small cutaneous growth of from 2 to 3 mm in size formed. The stump was fixed in 12% neutral formalin. The method of E. I. Rasskazova (a modification of the Bielschowsky method) was used to impregnate the nerve fibers. We were not able to detect visible morphological changes in the nerve fibers innervating the striated muscles, the connective tissue, and the skin of the stump. However, in the distal portion of the stump, growth of individual nerve fibers was observed, so strong that the nerve fibrils enveloped the nonregeneration ends of the muscle fibers in an unbroken net. The individual nerve fibers permeated the entire cutaneous protuberance which had formed on the wounded surface (Fig. 1, a, b).

The results of the experiments led to the notion that roentgen rays in the dose we used do not suppress the growth ability of nerve fibers. However, one could propose that the vigorous growth of the nerve fibers in the distal portion of the stump was pathological, representing a response to the action of radiant energy. The absence of visible morphological changes in the actual fibers still is not sufficient reason to conclude that the response is absent from the nervous system.

In our experiments, both the receptors and the effectors could be damaged as a result of irradiation, i.e., the whole terminal nervous apparatus of the extremity. In both cases, the normal nervous connections with the the center were changed. Allowing for such a possibility, we decided to denervate the irradiated extremity by sectioning the nerves of the lumbo-sacral plexus, in order to cause atrophy of the irradiated terminal nerve apparatus. Due to the regeneration of the nonirradiated nerve, the terminal nerve endings also must be restored. Thus, we could, so to speak, restore the peripheral section of the extremity's nervous system, replacing the irradiated peripheral nerve fibers and the terminal apparatus with nonirradiated regenerated ones.

If the loss of the regeneration ability in the irradiated portion is only a consequence of the injury to the peripheral nerve fibers and to the terminal apparatus, then the regeneration of new nerve fibers should restore this ability. In other words, when the irradiated portion of the limb is amputated after the regeneration of the nerve fibers, regeneration may be hoped for. Cutting through the nerve trunks produced somewhat higher branching places in the 16th, 17th and 18th spinal cord nerves (Fig. 2).

Denervation was carried out on 12 axolotls. The right irradiated extremity was denervated; the left irradiated extremity was used as the control (5,000 r dose). Irradiation was done two months before denervation. The denervated extremity lost the ability of independent movement and sensibility. It hung, like a whip-lash, and did not help the animal to swim. When the denervated extremity was pricked with the sharp end of pincers, the axolotl did not withdraw it, whereas the control extremity, when pricked, responded quickly to the irritation.

Restoration of motor and sensory fibers in the denervated organ proceeded rather slowly. The appearance of a sensory and motor reaction in the denervated irradiated extremities was observed only by the 50-70th day. In 4 axolotls, disintegration of the irradiated extremity tissues occurred due to denervation, and these animals were consequently excluded from the experiment. In the remaining 8 axolotls, when motion and sensibility were restored to the denervated extremity, simultaneous amputation of the experimental and control extremities

was done. During a period of four months after the amputation (observation period), regeneration of the amputated extremities did not occur.

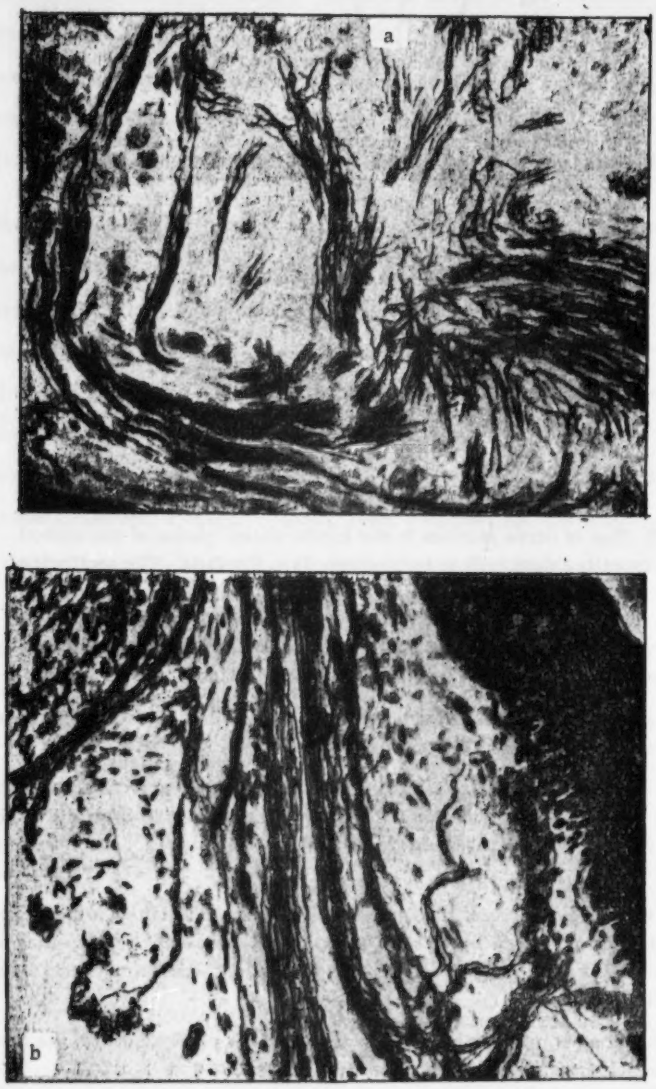


Fig. 1. Growth of nerve fibers in the stump of the irradiated extremity of an axolotl.
a) in the distal portion of the stump muscles; b) in the skin protuberance.

One could propose that not only the peripheral nervous system suffers from irradiation, but other higher orders of nervous connections, that provide for regeneration, as well (the spinal cord, the nerve ganglia). To prove this proposal, we set up the following experiment. The right rear extremity in 10 axolotls was denervated before irradiation. This, to a considerable extent, excluded the possibility of direct transmission of strong

stimulation (in the case of irradiation) to the central nervous system. The left rear extremity was not denervated, and was used as the control.

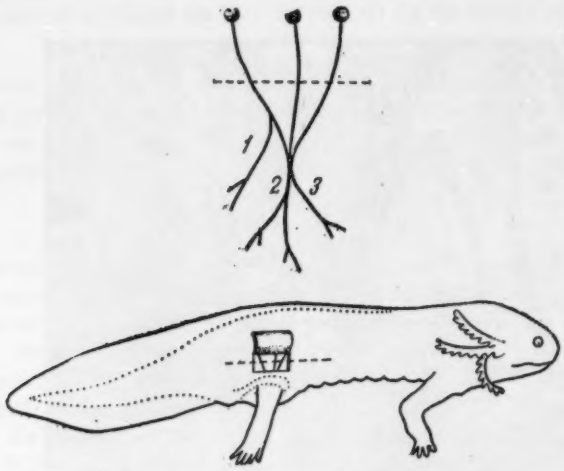


Fig. 2. Plan of nerve sections in the lumbo-sacral plexus of the axolotl. 1) n. cruralis anterior; 2) n. ischiadicus; 3) n. fibularis. The sectioning line is shown by the dots.

A week after denervation, both rear extremities of the axolotls were irradiated (5,000 r). Six days after irradiation, signs of tissue destruction in the denervated extremity began to appear in two of the axolotls.

In the remaining axolotls, restoration of the sensory and motor reactions of the right extremity occurred, just as in the preceding experiment, 50-70 days after denervation.

After this, both rear extremities of the axolotls were amputated at the distal third of the femur. In 12-14 days, epithelization of the wounded surfaces was noted on both extremities of all the axolotls. Organotypical regeneration was not observed in any of the axolotls during a period of three months after the amputation.

One could propose that, because of the way this experiment was conducted, we could not fully exclude the transmission of the stimulation, which occurred as a result of irradiation, to the central nervous system. Consequently, the regeneration ability was not restored, in spite of the sectioning of the nerves of the lumbo-sacral plexus before irradiation.

It is also possible that the loss of the regeneration ability is not connected with the disruption of nervous connections, but with some other changes of a different sort.

SUMMARY

It was demonstrated that the amputation of an irradiated organ does not bring about its regeneration in the case of the denervation of the axolotl extremity x-rayed with the dose of 5000 r.

Under the conditions of provisional denervation of the intact extremity followed by irradiation, and amputation after innervation restitution, no regeneration was found. The loss of regeneration ability in the axolotl extremity is, apparently, unconnected with the change of peripheral nerve fibers in the stump.

LITERATURE CITED

[1] V. V. Brunst, Uspekhi Sovremennoi Biol., 1937, Vol. 6, No. 3, pp. 469-498.

[2] K. F. Galkovskaya, Doklady Akad. Nauk USSR, 1951, Vol. 81, No. 5, pp. 945-948.

[3] K. F. Galkovskaya, Doklady Akad. Nauk, USSR, 1952, Vol. 87, No. 5, pp. 865-868.

[4] V. N. Zamaraev, Byull. Eksptl. Biol. i Med., 1939, Vol. 8, No. 6, pp. 535-538.

[5] G. S. Strelin, N. K. Shmidt, and Yu. D. Zilberg, Doklady Akad. Nauk, USSR, 1950, Vol. 75, No. 3, pp. 465-468.

[6] G. S. Strelin, Doklady Akad. Nauk, USSR, 1950, Vol. 73, No. 6, pp. 1283-1286.

[7] Yu. Yu. Shaksel and G. G. Shneider, Doklady Akad. Nauk, USSR, 1939, Vol. 23, No. 9, pp. 957-961.

[8] V. V. Brunst, Quart. Rev. Biol., 1950, Vol. 25, No. 1, pp. 1-29.

[9] E. G. Butler, J. Exptl. Zool., 1933, Vol. 65, No. 3.

[10] E. G. Butler and O. E. Schotte, J. Exptl. Zool., 1949, Vol. 112, pp. 361-392.

[11] W. O. Puckett, J. Morphol., 1936, Vol. 59, No. 1, pp. 173-208.

[12] O. E. Schotte and A. G. Karzmar, J. Exptl. Zool., 1944, Vol. 97, pp. 1-28.

[13] M. Singer and L. Craven, J. Exptl. Zool., 1948, Vol. 108, pp. 279-309.

THE ANTIBIOTIC PROPERTIES OF THE EJÁCULATES OF MAMMALS AND MAN

V. N. Kulikova

Dept. of Embryology (Chief – Prof. B. P. Tokin), Leningrad State University

(Received April 19, 1956. Presented by Prof. P. S. Kupalov, Active Member of the Academy of Medical Sciences, USSR)

The purpose of our work was to examine the secreta of the accessory genital glands of mammals and man for bactericidal and fungicidal properties.

EXPERIMENTAL METHODS

We used the sperm of chinchilla rabbits in the experiments. A series of experiments was conducted using human ejaculate. Bacillus subtilis, Micrococcus lysodeikticus, Staphylococcus aureus, Vibrio fluorescens, Torula utilis yeast and fungi from the Actinomycetaceae family, Actinomyces alba and Actinomyces griseus were used to determine bactericidal and fungicidal properties.

Rabbit sperm was received in an artificial vagina constructed according to V. K. Milovanov [1]. Human sperm was provided by the clinic of the Otto Gynecologico-Obstetrical Institute. The rabbit sperm was used in the experiment 10-15 minutes after it had been received; the human sperm, on the average, was used within 4 hours. Ejaculate with active spermatozoa was used to set up the experiment. Two experimental methods were used.

In the first method (I), the rabbit ejaculate was sterilized by being passed through a Seitz filter with an asbestos pad. First, however, the experimental fluid was diluted with 5 parts of water, since the amount obtained from two ejaculations (1-2 cm^3) was not enough for the rate of filtration. An emulsion of bacteria or fungi was added to the filtrate in a proportion of 2:1. In the experiments with bacteria, the emulsion contained 100,000 cells per 1 cm^3 – with fungi, 10,000 cells per 1 cm^3. Either a fluid favorable to microorganisms (meat-peptone bouillon or must) or sterilized water was used as the control. The experimental and control test tubes were placed in an incubator at a temperature of 37° (for the bacteria) or 27° (for the fungi). Immediately, and then in 1, 5 and 24 hours, the culture medium from the experimental and control test tubes was transferred to a solid medium in sectors by a platinum loop. The experiment was repeated 8 times. The results were determined after 24 hours (for bacteria) or after 48 hours (for fungi).

To verify the results obtained from the experiments with diluted rabbit ejaculate, we set up another series of experiments in which human sperm was examined by another method (II). 1.5-2 cm of a Petri dish was filled with a meat-peptone agar and then seeded with a one-day bacteria culture of 1,000 colonies transferred on to the dish by a pipette (with this method, no experiments with fungi were conducted). Then, under sterile conditions, a heated, sterile glass "ring" (cylinder), 0.6 cm in diameter was lightly fixed to the agar. 0.5 cm^3 of the undiluted sperm or ejaculate, which had previously been stripped of spermatazoa by centrifugation in order to exclude the possibility of bactericidal action from the substances secreted by the spermatazoa during their activity, was dropped into the ring. As the control, meat-peptone, bouillon was used, or an empty ring was fixed in order to find whether the ring temperature influenced the bacteria. The experimental and control dishes containing the cultures were placed in an incubator at 37°. The results were calculated after 24 hours.

EXPERIMENTAL RESULTS

The secreta of the accessory genital glands of the rabbit are destructive to V. fluorescens, A. alba and A. griseus (see table). Even after an hour's exposure, the growth of the Actinomycetes colonies was noticeably weaker than in the control. An average of about 100 colonies grew up in the control; in the experiment, about 10. The cultures showed no growth whatsoever after 5-24 hours. From this one can conclude that the secreta of rabbit accessory sex glands have strong fungicidal properties with respect to the Actinomycetes used.

Action on Different Microorganisms of the Accessory Genital Gland Secreta of the Rabbit

Microorganism	Number of rabbits	Number of experiment		Bactericidal and fungicidal properties discovered		No bactericidal or fungicidal properties discovered	
		I	II	I	II	I	II
B. subtilis	7	35	10	2	—	33	10
M. lysodeikticus	7	46	10	—	—	46	10
S. aureus	7	42	2	—	—	42	2
V. fluorescens	6	27	2	18	2	9	—
T. utilis	3	17	--	—	—	17	—
A. alba	3	27	—	27	—	—	—
A. griseus	3	27	—	27	—	—	—

Note: I) experiments set up according to the first method; II) experiments set up according to the second method.

In the experiments with the action of the fluid portion of the rabbit ejaculate on V. fluorescens, it was discovered that not only the ejaculate from different males, but even different batches from the same male act in different ways upon this microorganism. The feeding and maintenance conditions of the experimental animals and the experimental processes were identical. Eighteen out of 27 experiments conducted by the first method had a bactericidal effect. Only 2 experiments were done with the second method; in both cases bactericidal action resulted.

The secreta of the accessory genital glands of male rabbits were found to have no bactericidal properties with respect to M. lysodeikticus, B. subtilis, S. aureus, or fungicidal properties with respect to T. utilis.

S. aureus and M. lysodeikticus were used to study the bactericidal properties of human ejaculate. The ejaculate of 20 men was examined — in 11 cases it was normal; spermatozoa quantity was low (up to 10 specimens per visual field) in 4 cases, and azoospermia was found in 5 cases. The amount of experimental fluid (1-3 cm^3) was not always sufficient to permit experiments with both kinds of bacteria. Twenty experiments were done with Staphylococcus aureus, in 10 of these a sterile zone 0.2-0.5 cm in size was established around the ring affixed to the agar. S. aureus did not grow inside the ring in any case, including the one experiment in which azoospermatic ejaculate was used. Twelve experiments were done with M. lysodeikticus — in two a bactericidal effect was discovered.

The human ejaculate was not sterilized. In the course of culturing the media, different bacteria of a common aerial microflora and other specific bacteria were discovered in it. Special tests established that these were not antagonistic to S. aureus and M. lysodeikticus. Consequently, their presence had no influence on the results of the experiments.

Since the experiments were done in vitro, one cannot state that the experimental fluid would have the same properties in the body. Moreover, the microorganisms were chosen arbitrarily.

SUMMARY

Fungicidal and bactericidal properties of the secreta of the accessory genital glands in mammals and man were studied.

It was found that the secreta of the accessory genital glands in rabbits possess strong fungicidal properties with respect to Actinomycetes (A. alba and A. griseus) and to V. fluorescens.

The growth of colonies in the experiments was ten times less than that in the control.

Human ejaculate possesses bactericidal properties with regard to Staphylococcus aureus.

LITERATURE CITED

[1] V. K. Milovanov, Artificial Insemination of Agricultural Animals, Moscow 1938.*

* In Russian.

THE FUNCTIONAL SIGNIFICANCE OF THE INNERVATION OF THE HUMAN UMBILICAL CORD

V. N. Blyumkin

Dept. of Histology (Chief – Assistant Prof. V. N. Blyumkin), Vitebsk Medical Institute
(Director – Assistant Prof. I. I. Bogdanovich)

(Received September 25, 1956. Presented by Prof. V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR)

The gelatinous tissue of the human umbilical cord is not only important to the fulfillment of the supporting-mechanical function. There are reports in the literature indicating that there are biologically active substances present in Wharton's jelly.

K. Ko [11] noticed that the biological action of the extract from the umbilical cord differed from the action of placental extracts. According to E. O. Ryabushko and M. M. Kholodenko [9], umbilicaline (the extract from the umbilical cord) in dilutions of 1:100-1:500 causes some intensification and frequency increase in the action of an isolated frog heart. K. M. Voloshina [3] found a sympathicotropic action of umbigen (water-alcohol extract from dried blood extracted from the umbilical vessels) on the anuclearized eyes of frogs. When umbigen was administered parenterally to rabbits, an increase in the sugar content of the blood was observed. N. S. Kharenko and R. I. Sharlai [10] reported on the content of definite amounts of dehydroadrenalin and acetylcholine in umbigen.

The purpose of this work was to compare the data regarding the content of biologically active substances in Wharton's jelly with the results of morphological study, particularly with the data on the innervation of the human umbilical cord gelatinous tissue.

EXPERIMENTAL METHODS AND RESULTS

Neurological and histological studies in recent years [1, 2, 4, 5, 6, 8] have examined in detail the innervation of the proximal portion of the human umbilical cord.

Using a supravital stain of methylene blue according to A. S. Dogiel and silver impregnation according to Kampos, we established that the penetration depth of single nerve fibers into the mature umbilical cord consisted of 12-20 cm from the umbilical ring. In Wharton's jelly, small ligneous endings and many primitive terminal apparatuses shaped like small loops and plates were found. The ligneous endings with the terminal plates did not contact the fibroblasts of Wharton's jelly, whereas the endings of the more primitive structures could be distributed both along the intercellular interstices and in the cytoplasm of the fibroblasts.

The delicate structure of the fibers innervating Wharton's jelly was completely heterogeneous. They all were nonmyelinated, but one could distinguish four varieties of these fibers. The first variety of fibers was flat and had a distinguishable neurofibrillar structure and a medium amount of neuroplasm. The second sort of fiber were axons, rich in neuroplasm and having bulges. The third kind of fiber was characterized by the formation and splitting off of neuroplasm drops in varying sizes. In accord with N. D. Zaitsev [4], we treated this process as an unusual neurosecretion. Finally, the fourth variety of fibers were axons in a state of destruction similar to wallerian degeneration. The axons with the signs of neurosecretion and degeneration were especially interesting.

In neurosecretion, spheroid, neuroplasmic protuberances could form. From a separate axon, or from one entering the body of a trunk, a neuroplasmic protuberance of 20 microns or more in diameter was formed. At first, this protuberance was connected with the axon engendering it, then the neuroplasmic stalk was resorbed and the substance of the spheroid protuberance gradually formed into an argentophilic drop, situated in the intercellular substance of Wharton's jelly. Then the drop was gradually resorbed into the gelatinous tissue.

As well as the large neuroplasmic protuberances which formed, many small drops could split off from the axon also, subsequently being resorbed into the interstitial substance of the umbilical cord gelatinous tissue (Fig. 1).

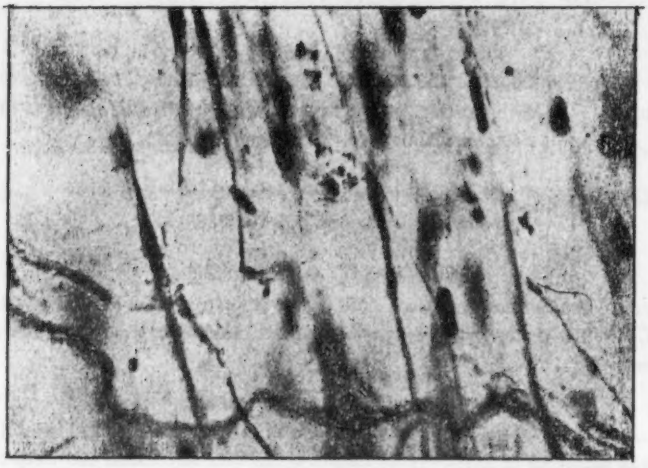


Fig. 1. Innervation of the gelatinous tissue of a mature human umbilical cord. The terminal branches of the axons are seen. Neurosecretion. Many small drops of neuroplasm secreted. Silver nitrate impregnation according to Kampos, Magnification 99. Immersion.

Many of the nerve fibers were in a process of physiological destruction similar to wallerian degeneration.

The neurosecretion and destruction processes of the nerve fibers increased during the second half of uterine development. In connection with this, it is interesting to note that, in studying the histogenesis of the gelatinous tissue of the human umbilical cord, M. Ya. Levina [7] found secretory processes occurring in the fibroblasts, and also destructive changes in the sedentary cells of Wharton's jelly, and these were also most sharply expressed during the second half of embryogenesis.

Since there were no nerve fibers discovered in the space 18-20 cm from the umbilical ring, we provisionally divided the umbilical cord of the human embryo into an innervated zone and a zone with no apparent innervation. Based on morphological observations, one can suggest that there is also a physiological difference between the proximal and distal sections of the umbilical cord. To prove this hypothesis, we studied the effect of extracts from the two sections of the umbilical cord on an isolated frog heart.

For the experiments, we used only the nonvascular portions of the umbilical cord, since the umbilical cord vessels are connected with the placenta, the extracts of which contain acetylcholine. The umbilical cord was cut into small pieces with scissors, ground into a pulp in a porcelain mortar, inundated with Ringer's solution in an amount of nine times its weight, carefully blended and extracted over a period of several hours. The pulp was then pressed through cloth filters. We considered the extracts obtained to be 10% solutions.

The activity of the extracts was tested on a frog heart, isolated according to Shtraub. More than 500 extracts from 48 umbilical cords of mature human embryos were tested. Physiological testing of the extracts was carried out with the appropriate morphological control.

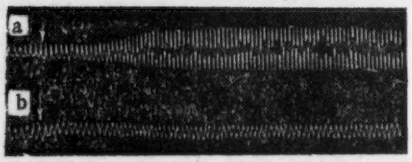


Fig. 2. Change in the action of an isolated frog heart during the action of the extract. a) extract from the proximal section of the umbilical cord; b) extract from the distal section. Dilution 1:100. The arrow shows the moment at which the extract was injected.

Figure 2, a, gives the recording of the action of the isolated frog heart exposed to the action of the extract from the proximal section of the umbilical cord. The kymogram shows the clear, positively inotropic effect. The rhythm of the heart action grew considerably faster. The extract took effect swiftly and the effect was observed in a series of experiments to last 10 minutes or longer (without washing by Ringer's solution). Thus, the use of the extract from the proximal portion of the umbilical cord caused an effect of a sympathomimetic character.

The extract from the distal portion of the same umbilical cord in the same dilution had only a slight effect on the isolated frog heart (Fig. 2, b).

The contraction amplitude even decreased slightly at first, and only afterwards returned to the initial level; the increase in the frequency of the cardiac contractions was considerably less than with the action of the extract from the proximal portion of the umbilical cord.

In some experiments, the extract from the distal potion of the umbilical cord caused only a gradual and very slight, as compared with the action of the extract from the proximal portion (Fig. 3, a), intensification of the cardiac contractions with a simultaneous slight frequency increase (Fig. 3, b).

Extracts from the middle portion of the umbilical cord caused a slighter adrenalin-like effect than extracts from the proximal portion, but a greater one than extracts from the distal portion. The rule was established for 1:100-1:5,000 dilutions of the extracts. Maximal efficiency of the extracts was observed with a dilution of from 1:100 to 1:200. Thus, the grade of sympathomimetic activity of the umbilical cord extracts decreased from the umbilical ring towards the placenta. Obviously, the problem is how the biologically active substances with the adrenalin-like action in the proximal section of the human umbilical cord are formed.

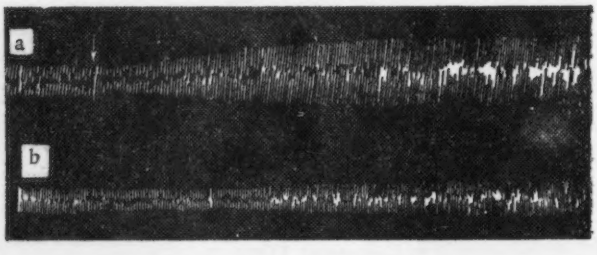


Fig. 3. Change in action of isolated frog heart due to the action of the extract.
a) extract from the proximal portion of the umbilical cord;
b) extract from the distal portion. Dilution 1:100. The arrow shows the moment at which the extract was injected.

In searching for the morphological expression of this formation process, we checked at the secretory-like phenomena – the formation of the neuroplasm drops which split off from the axons innervating Wharton's jelly, and the processes which M. Ya. Levina [7] described by which the gelatinous substance is formed.

In order to decide which of the above-mentioned morphological phenomena are most connected with the varying physiological action of the extracts from the different sections of the umbilical cord, we studied the question of the distribution of the secretion phenomena fibroblasts (according to M. Ya. Levina) in the proximal, middle and distal sections of the umbilical cord. No quantitative predominance of the secreting sedentary cells of Wharton's jelly in the proximal section, as compared with the distal, was found. When this fact is compared with the discovery of nerve fibers only in the first third of the umbilical cord, the hypothesis follows that the nerve fibers which innervate Wharton's jelly actively participate in the production of the biologically active substances with the adrenalin-like action.

It is possible that the formation of such substances is in some measure connected with the phenomena of mass drop-formation and destruction of the nerve fibers. Morphological data confirm this idea. In cases when a sharper sympathomimetic effect on the isolated frog heart was noted in the physiological experiments, silver impregnation showed a clearer expression of the neurosecretion and physiological nerve fiber destruction processes. Thus, it seems that the biological activity of the extracts from the proximal portion of the umbilical cord is also connected with the chromaffin paraganglia, which we recently discovered, localized in the region of the umbilical ring, along the path of the intra-abdominal part of the umbilical arteries and on the expanse of the first 5-6 cm of the umbilical cord. The problem of the paraganglia in the umbilical ring region will be treated in a special report.

Extracts prepared from the Wharton's jelly of the innervated portions of the umbilical cord show a positive chronotropic or inotropic action on the isolated frog heart. The extracts from the distal portions give a much weaker action. The descending morphological grade of umbilical innervation also corresponds to the descending sympathomimetic activity grade of the extracts from the different sections of the umbilical cord. A direct relation between the innervation of the umbilical cord and the biological activity of its extracts can be suggested.

SUMMARY

The morphological investigation of Wharton's jelly of the human umbilical cord led to the discovery of single nerve fibers in 12-20 cm from the umbilical ring. Many of the fibers which innervate the jelly tissue of the umbilical cord show signs of neurosecretion.

LITERATURE CITED

[1] V. N. Blyumkin, Doklady Akad. Nauk, USSR, 1956, Vol. 106, No. 1, pp. 133-135.

[2] V. N. Blyumkin, Program of the Scientific Session dedicated to the 85th Anniversary of the V. I. Lenin Vitebsk Medical Institute and Abstracts of the Proceedings,* Vitebsk, 1955, pp. 25-25.

[3] K. M. Voloshina, in the same work [2], pp. 233-237.

[4] N. D. Zaitsev, in the book: Works of the 5th Meeting of Anatomists, Histologists and Embryologists,* pp. 603-606, Leningrad 1951.

[5] V. G. Kaminskaya, Akush. i Ginekol. 1954, No. 2, pp. 54-58.

[6] V. G. Kaminskaya, Byull. Eksptl. Biol. i Med 1954, No. 7, pp. 73-77.

[7] M. Ya. Levina, The Development of Gelatinous Tissue in the Human Umbilical Cord,* Dissertation, Leningrad 1949.

[8] S. A. Petrova, in the book: Abstracts of the Proceedings of the Scientific Conference of the Ryazansky Medical Institute,* pp. 37-46, Ryazan, 1953.

[9] E. O. Ryabushko and M. M. Kholodenko, in the book: New Preparations for Tissue Therapy,* Kiev 1952, pp. 269-275.

[10] N. S. Kharchenko and R. I. Sharlai, Vrachebnoe Delo, 1950, No. 10, pp. 925-928.

[11] K. Ko, Japan J. Med. Sci., IV – Pharmacology, 1935, Vol. 9, No. 1, pp. 1-28.

* In Russian.

THE CONTENT OF ACETYLCHOLINE-LIKE SUBSTANCES AND CHOLINESTERASE IN THE TISSUES OF THE ASCARIS

A. I. Krotov

From the Institute of Malaria, Medical Parasitology and Helminthology of the Ministry of Health Preservation, USSR (Dir. – Prof. P. G. Sergiev), Moscow

(Received January 17, 1956. Presented by Prof. P. G. Sergiev, Active Member of the Academy of Medical Sciences, USSR)

To date, acetylcholine and cholinesterase have been found in the tissues of all free-living invertebrates which possess a developed nervous system. They have been found in a series of animals which lead a parasitic form of life. M.P.A. Chance and T. E. Mansour [6] established the presence of real cholinesterase in the trematode Fasciola hepatica. N. M. Artemov and R. N. Lurye [1] discovered acetylcholine and cholinesterase in the tissues of cat cestodes. N. M. Artemov [1] found cholinesterase in parasitic crustacea. In the ascaris, E. Baldwin and V. Moyle [5] could not find acetylcholine or cholinesterase, and expressed the opinion that they were absent.

However, acetylcholine was recently discovered in the tissues of the ascaris by M. Helen [7].

The method we used to register the spontaneous movements of whole ascarides [2, 3] allowed us to establish their extremely high sensitivity to acetylcholine, especially after the preliminary action of proserine.

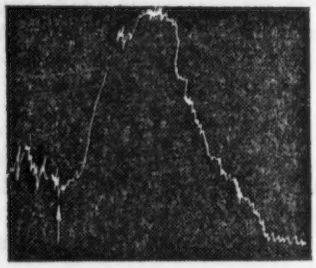


Fig. 1. Tetanic contraction of an ascaris in a solution of proserine, caused by acetylcholine in a dilution of 10^{-20}. The arrow shows the moment the preparation was injected.

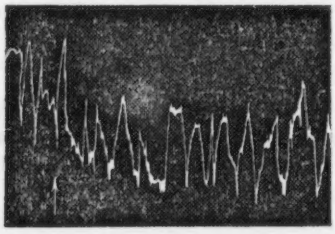


Fig. 2. Slight debilitation of an ascaris body, caused by a total solution of another ascaris in a dilution of 1:100. The arrow shows the injection as in Fig. 1.

The ascarides responded by a sharp body contraction when acetylcholine in a dilution of up to 10^{-11} or proserine in a dilution of up to 10^{-9} was applied to their lips or caudal ends. After the preliminary action of proserine in

Fig. 3. Contracture of the dorsal muscle of a leech, caused by a total solution of an ascaris previously immersed in a solution of proserine in a dilution of 10^{-12}. Arrow the same as in the other figures.

Fig. 4. Tetanic contraction of an ascaris, caused by a total solution of another ascaris, previously immersed in a solution of proserine in a dilution of 10^{-12}. Arrow the same as in other figures.

an inactive concentration (10^{-15}), acetylcholine caused an answering reaction in the ascaris in dilutions of 10^{-20}-10^{-21} (see Fig. 1). Ascarides in which the lips and tails had been removed, and also ascarides in solutions of novocaine, stopped reacting to acetylcholine and proserine [4].

Skin-muscle preparations of ascarides reacted to acetylcholine in a dilution of 10^{-6}, and to proserine in a dilution of 10^{-5}.

In order to determine the presence of acetylcholine and cholinesterase in the ascaris more exactly, we tested the effect of a total solution of an ascaris and of an extract from ascaris skin-muscle sheaths on the isolated dorsal muscles of a leech, and also on live ascarides. These preparations had no visible influence on the dorsal muscles of the leech, and caused only a slight debilitation in the ascaris (Fig. 2).

Consequently, the ascaris extracts did not contain specific acetylcholine-like substances.

We also used a total solution of an ascaris and extracts from the ascaris skin-muscle sheath, which were prepared after the live ascarides had been immersed in a solution of proserine for 2 hours previously (dilution 10^{-12}). Such preparations diluted up to 10^{-3} caused sharp contractures of the dorsal muscles of the leech and tetanic contractions of the ascaris in a dilution of 10^{-4} (Fig. 3-4).

In order to detect cholinesterase, we added different amounts of the ascaris tissue extracts to acetylcholine in a dilution of 10^{-7}. Five hours after preparation, these solutions were tested on the proserine-processed dorsal muscles of a leech. The total ascaris solution completely inactivated acetylcholine in a dilution of 1:10, the extract from the cerebral ganglia in dilutions up to 1:1,000 and the lip extracts in dilutions up to $1:10^{-5}$. One can conclude from these experiments that cholinesterase is present in the tissues of the ascaris, primarily in the sensory endings, but also in the nerve cells.

SUMMARY

Registration of spontaneous movements of whole ascarids have shown that the nerve endings of their lips and tails are highly sensitive to acetylcholine. Previously applied proserine considerably increases their sensibility. Biological tests on the dorsal muscles of the leech and on ascarids proved the presence of acetylcholine-like substances and cholinesterase in ascarids.

LITERATURE CITED

[1] N. M. Artemov and R. N. Lurye, Bull. Acad. Sci. USSR, 1941, No. 2, pp. 278-282.

[2] A. I. Krotov, Med. Parazitol. 1953, No. 5, pp. 387-395.

[3] A. I. Krotov, Med. Parazitol., Vol. 25, No. 1, pp. 58-60.

[4] Ibid., pp. 60-62.

[5] E. Baldwin and V. Moyle, Brit. J. Pharmacol. 1949, Vol. 4, No. 2, pp. 145-152.

[6] M. P. A. Chance and T. E. Mansour, Brit. J. Pharmacol. 1953, Vol. 8, No. 2, pp. 134-138.

[7] M. Helen, Parasitol., 1954, Vol. 45, No. 3-4.

MORPHOLOGY AND PATHOMORPHOLOGY

CHANGE IN THE TRANSITIONAL EPITHELIUM IN THE CASE OF REPARATIVE REGENERATION

M. P. Ptokhov

From the Laboratory of Experimental Morphology (Head – Prof. N. G. Khlopin, Active Member, Academy of Medical Sciences, USSR), the Institute of Oncology (Director – Prof. A. I. Serebrov, Corresponding Member of the Academy of Medical Sciences, USSR), Academy of Medical Sciences, USSR, Leningrad

(Received June 8, 1956. Presented by N. G. Khlopin, Active Member of the Academy of Medical Sciences, USSR)

The study of epithelial reparative regeneration brought us to the problem of how wounds heal. Epidermal regeneration has been the facet of this problem most completely studied. But there are very few works concerning regeneration of the transitional epithelium [4, 11, 14, 16]. The experimental works of recent years that have been done on the transitional epithelium have especially not posed or resolved the problems of regeneration [1, 2, 5, 7, 8, 9, 15].

N. G. Khlopin and his colleagues have shown that the epidermal tissues are stratified and vertically anisomorphic. The transitional epithelium is normally a stratified, polyserial, mucous-producing sheet. Under experimental conditions, in tissue cultures, it produces true stratified structures and even forms intercellular bridges [5, 6].

The purpose of this work was to study certain features of reparative regeneration in the bladder epithelium, proceeding from recent ideas concerning the epithelial tissues.

EXPERIMENTAL METHODS

The work was done on 28 rabbits. The celiac cavity and the bladder were opened by operation. The mucous membrane of the bladder was cauterized in 4-5 places with a red-hot metal plate, or else small sections were cut from it. The damage area was from 4 × 4 to 6 × 6 mm. As a rule, the underlying connective tissue was injured as well as the epithelial sheath, and, in some cases, the musculature of the bladder was also damaged.

The animals were sacrificed at intervals ranging from 12 hours to 15 days after the operation. The bladder was fixed while distended and processed for histological examination. We prepared sections stained with hematoxylin-eosin, iron hematoxylin, mucicarmine and eosin-azure, and also flattened, total preparations. These last were stained with iron hematoxylin according to a method of our own [5].

EXPERIMENTAL RESULTS

Scabs formed immediately on the seared places. During the first hours, dystrophic and necrobiotic changes predominated on the epithelial layer, along the edge of the burn and for some distance from it – vacuolization of the cytoplasm, abnormal nuclei, etc. In the connective tissue underlying the scab and for some distance from it, a marked inflammatory reaction was observed. The vessels became dilated, edema developed, inflammatory leucocytic infiltrations and small foci of hemorrhage appeared.

After 12 hours, the first mitoses appeared in the basal cell layer, receding a little from the edge of the burn. Their number had sharply increased after 24 hours. The mitotically dividing cells were rounded. Later on, the number of mitoses continued to increase, and dividing cells were found in the basal and middle cell layers. The volume of the cells and nuclei noticeably increased. Among the basal elements, one could see leucocytes and macrophages infiltrating from the connective tissue. Erthrocytes were also found, but they occurred more often in small masses.

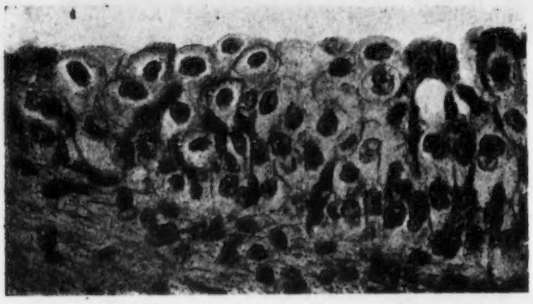


Fig. 1. Regeneration of the bladder epithelium 2 days after the burn. Burn located on left. Marginal portion of regenerate. The structure of the epithelial layer, becoming simplified, has lost the trizonal structure characteristic of the transitional epithelium. Mitosis. Stained with hematoxylin-eosin. Magnification 320.

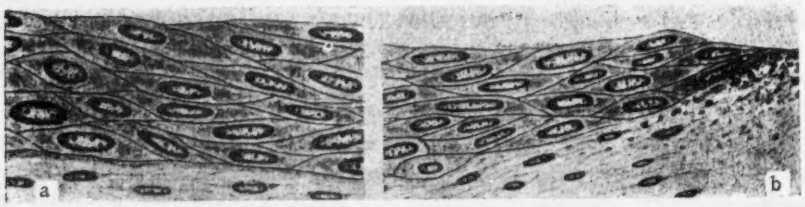


Fig. 2. Regeneration of the bladder epithelium 3 days after the burn. Epithelial layer has become multilayered in structure. The cells of all the layers are compressed and do not show visible difference of differentiation. Magnification 400. a) regenerate portion some distance from edge; b) edge portion of regenerate.

The death and sloughing of the differentiated cells of the superficial layer, as well as some of those of the middle layers, and the vigorous multiplication of the basal elements led to change in the structure of the layer. Its cell elements became more or less uniform and basophilic. The vertical anisomorphic character disappeared. The epithelial layer became less differentiated (Fig. 1).

By the end of the third day, there was a marked epithelial advance towards the damage. An epithelial thickening formed around the scab. One could see on vertical sections that, in the marginal portions of the epithelial regenerate, the cells of all the layers were compressed and oriented towards the direction in which the epithelium was moving, and that their horizontal dimensions had become considerably more vertical. The epithelium moved forward along the developing granulation tissue. In these places, the epithelium acquired the structure of a poorly differentiated, multilayered epithelium with 6-9 layers, with no expressed vertically

anisomorphic character; the cells of all the layers became compressed (Fig. 2). The epithelial regenerate, coming across the scab, either formed an incrustation and became considerably thicker, or, moving up under the scab, became wedge-like in shape (in cross-section).

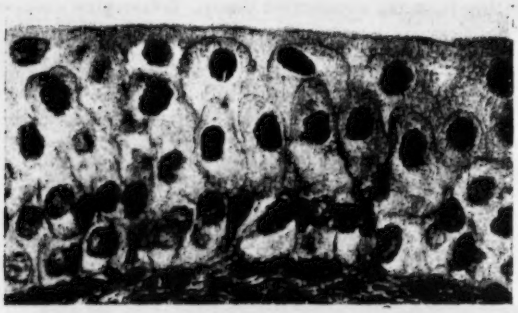


Fig. 3. Regeneration of the bladder epithelium 12 days after burning. Redifferentiation of the regenerate. The layer has again acquired the structure of the transitional epithelium. Stained with hematoxylin-eosin. Magnification 465.

The regenerating transitional epithelium approached the damage in a multilayered sheet. As N. A. Shevchenko [10] has shown, such a method of regeneration is characteristic of the cutaneous type of epithelia. Thus, this feature also emphasizes that the transitional epithelium belongs to the tissues of the epidermal type.

In a series of cases, moreover, buried inflammatory growths of the epithelium were observed. It grew deep inside, like massive rods. Sometimes, one could see a group of cells which had separated from the rod next to it. Such cell complexes became spherical in form. They have been described by A. Brunn [12], and are known in the literature as "Brunn's nests." When a foreign body was placed in the rabbit bladder, we observed buried inflammatory growth of the epithelium to develop. In this case, structures which are known in human pathology as cystic and glandular cystitis [7] also formed. R. Giani [15] also observed the buried development of the transitional epithelium with the formation of cysts.

In some places, during the wounded stages of the regenerative process, erythrocytes and lumps of detritus were seen in the surface layers of the epithelial sheet, between and inside the cells. Sometimes they were piled up, like coins, in columns. These epithelial cells replete with erythrocytes and heaps of detritus were later sloughed. The presence of erythrocytes in the cells of the transitional epithelium has been described by A. V. Cornil and P. Carnot [14] and by A. S. Lezhava [3]. By the 6th-8th day, the newly-formed epithelial sheet had already covered a considerable portion of the injury. In those places where the scab still remained, the advancing epithelium scaled it off.

In those cases where the mucous membrane had been cut, the picture was generally the same as that described above. The picture differed in that the scab was smaller, the injury more quickly covered over, the inflammatory symptoms were less marked and there were no dystrophic changes in the cells.

In the case of the burns, the scab usually fell off on the 10th-12th day, and the whole region of the former injury was sealed by the newly-formed epithelial sheet at about the same time. The place of the burn could be recognized by unevenness in the mucous membrane and by a stellate scar.

Mucous formation in the transitional epithelial sheet of the rabbits was usually weakly expressed. On the preparations stained with mucicarmine, drops of mucous could be seen in the cells of the interstitial and integumentary zones. The cytoplasm of the cells in the integumentary zone became a diffused light pink shade. In the regenerating portion of the transitional epithelium, the formation of mucous was slightly greater, and more drops of mucous were observed. In the marginal portion of the regenerate and in the epithelial incrustation

which abutted against the scab, cells were often found containing many small drops of mucous in their cytoplasm.

On the vertical sections, one could trace in detail those changes in the newly-formed epithelial sheet which were connected with the onset of redifferentiation. In its youngest portion (the most central), the cells of all the layers were compressed and arranged in 4-5 layers. The epithelial sheet was considerably thinner here than in the peripheral part of the former injury. The thickening of the sheet towards the periphery was due to the increased number of cell rows and to the increase in the vertical dimensions of the cells and to their changed shape. From being compressed and parallel to the surface, they became isoprismatic and then highly prismatic. They became more clearly defined, and the cell zones became more evident. The large cells of the integumentary zone were the largest cellular elements in the sheet. There were some polynuclear elements in the sheet. The cells of the interstitial and basal zones resumed their proper forms; basal branches appeared in them, and their apical ends became thickened and rounded off. The number of mitoses decreased at about this time (Fig. 3).

The changes in the epithelial sheet were closely associated with the character of the changes in the underlying connective tissue. The exudative phase of inflammation was replaced by the proliferative. The epithelial sheet advanced along the poorly differentiated granulation tissue. According to the stage of maturity, a loose binding of collagenous bundles was formed in it. On the 15th-16th day, the damaged portion of the bladder mucous membrane was more or less completely restored.

Our data, as well as the data of other experimental works studying the transitional epithelium give a sufficiently complete characterization of this tissue.

Normally a stratified-polyserial, mucous-producing, vertically anisomorphic sheet in the rabbit, the transitional epithelium loses its characteristic trizonal structure during the process of regeneration. The structure of the sheet becomes simplified and, in the marginal portions of the regenerate, poorly differentiated and multilayered. The cells of all the layers of such an epithelium are compressed. In this case, we are not dealing with metaplasia, but, according to the terminology of V. G. Garshin, with transitory changes connected with the processes of inflammation and regeneration. Later, during redifferentiation, the regenerate again acquires the structure natural to the transitional epithelium.

Therefore, in the case of transformations under experimental conditions, the transitional epithelium not only shows certain general histotypical properties characteristic to the epidermal type of tissue, but also retains to a known degree its own specific features, by which one can distinguish it from other related tissue of the epidermal type , during growth and proliferation.

SUMMARY

Studies of the reparative regeneration of the mucous membrane epithelium in the urinary bladder after a restricted burn or extirpation have revealed certain peculiarities of this process. The data obtained show that the transitional epithelium has under the conditions of regeneration certain histotypical properties characteristic of the epidermal tissue in general.

LITERATURE CITED

[1] M. A. Zakharyevskaya, Arkhiv Biol. Nauk, SSSR, 1938, Vol. 51, No. 3, pp. 80-93.

[2] A. S. Lezhava, Arkhiv. Biol. Nauk 1935, Vol. 37, No. 3, pp. 679-708.

[3] A. S. Lezhava, Soobshch. Akad. Nauk Gruz. SSR,1947, Vol. 8, No. 1-2, pp. 47-55.

[4] N. V. Petropavlovskaya, in the book: Works of the Leningrad Institute of Hygiene and Sanitation.* Leningrad 1953, Vol. 16, pp. 151-159.

[5] M. P. Ptokhov, Arkhiv Anat. Gistol. i Embriol., 1940, Vol. 23, No. 1-2, pp. 69-85.

[6] M. P. Ptokhov, Arkhiv Anat., Gistol. i. Embriol., 1940, Vol. 23, No. 1-2, pp. 53-68.

* In Russian.

[7] M. P. Ptokhov, Voprosy Onkology, Moscow, 1949, No. 1, pp. 204-211.

[8] N. G. Khlopin, General Biological and Experimental Foundations of Histology,* Leningrad 1946, p. 490.

[9] I. A. Chagirov, Vestnik Kazakhsk. Fil. Akad. Nauk SSSR, 1946, No. 2-3, pp. 61-64.

[10] N. A. Shevchenko, Arkhiv Anat., Gistol. i Embriol. 1941, Vol. 26, No. 1, pp. 97-128.

[11] A. Belzow, Arch. f. pathol. Anat., 1894, Bd. 97, S. 279-288.

[12] A. Brunn, Arch. mikroskop. Anat., 1893, Bd. 41, S. 294-302.

[13] N. Chlopin, Arch. f. pathol. Anat., 1924, Bd. 252, S. 748-773.

[14] A. V. Cornil and P. Carnot, Arch. med. experim. 1898, vol. 10, pp. 779-815.

[15] R. Giani, Beitr. z. path. Anat. u. allg. Pathol. 1907, Bd. 42, H. 1, S. 1-21.

[16] G. Lasio, Arch. f. pathol. Anat. 1904, Bd. 178, S. 65-82.

* In Russian.

DURATION OF THE EFFECT OF MEDICAMENTAL SLEEP ON INFLAMMATION

E. D. Klimenko

From the Laboratory of Pathomorphology (Head - Prof. A. A. Solovyev, Corresponding Member of the Academy of Medical Sciences, USSR), Institute of Normal and Pathological Physiology (Director - V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR), Academy of Medical Sciences, USSR, Moscow

(Received March 3, 1956. Presented by V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR)

The character of the morphological changes caused by the inflammatory process in different functional conditions of the central nervous system has not yet been made sufficiently clear.

Many of the available sources of literature indicate the inhibiting influence of medicamental sleep on the local inflammatory process [2, 5, 9, 11, etc]. Studies have been conducted on animals, in which prolonged sleep is induced by repeated injections of narcotic agents, and then the action of various stimulants studied on this background. These researchers were interested in the effect of the actual sleep, and did not take into account any condition which might be retained or which might occur after the sleep had passed. We did not find any data in the literature regarding the duration of medicamental sleep's influence on the reactivity of animals, although such a problem must have arisen in the course of medicamental sleep therapy.

The purpose of our work was to investigate the character and duration of the inflammatory reaction at different time intervals after medicamental sleep. The stimulants we used to bring about aseptic inflammation were peach oil and rat's fat. Besides the special interest that these substances had for us in connection with other work we were doing, we considered that the duration of the reaction processes caused by the subcutaneous injection of fatty substances would be useful in solving the given problem.

Many researchers have studied the morphology of the inflammatory reaction which is caused by the injection of fatty substances into tissue (the so-called oleogranuloma) – W. von Leube, 1895; Winternitz, 1903; V. Henderson and E.F. Crofutt, 1907; Henschen, 1914, V. V. Lauer [6], T. A. Pisareva [8], I. P. Vinogradov [3], G. A. Mokshanova, 1949, R. S. Braude and S. M. Gitman [1], and others.

The results of their works showed that subcutaneous injection of fat causes an inflammatory reaction similar to that caused by the injection of foreign bodies. The granulation tissue which arises as a result of this forms a nodule containing the injected substance, which divides into smaller nodules until, finally, the fatty substance is fully resorbed and replaced by scar connective tissue, sometimes with calcification. The speed with which the fat is resorbed depends on its derivation; according to the available data, vegetable fat is more slowly resorbed than animal fat (G. Brissy, Z. Binet and J. Verne; Veyrieres and Huerre).

EXPERIMENTAL METHODS

Our experiments were done with 70 white rats, weighing from 130 – 140 grams. The animals were fed the usual vivarium diet.

In each experimental series, one group of animals was injected with peach oil, the other with rat's fat (dose – 0.2 ml).

In the first experimental series, these substances were subcutaneously injected into the left flank 3 days after the sleep had passed. In the second series, the rats were injected after 7 days, and in the third, after 14 days. In all of the series, the morphological picture was examined on the 3rd, 7th, and 14th days, and, in a series of cases, also 24 and 48 hours after the fat injection.

We used a 2.5 solution of Barbamyl in a physiological solution as a hypnotic: 0.3 ml of this solution was injected subcutaneously into the rat's side at the level of the VIII – IX thoracic vertebrae. The hypnotic dose received caused sleep of varying durations, but these differences in duration were not reflected later outwardly in the general condition, behavior and weight of the animals.

We studied animals in which sleep had lasted from 2 hours to 4 hours 20 minutes. The microscopic material was fixed in a 12% solution of neutral formalin. The sections were stained with hematoxylin-eosin, and with sudan III for the fat.

EXPERIMENTAL RESULTS

The Morphological Picture in the Control Animals (not injected with the hypnotic). The first three days after the oil injection, the main mass of the oil concentrated in the central portions of the subcutaneous cellular tissue in nodules of different sizes, closely adjoining each other and surrounded by a considerable accumulation of polymorphonuclear leukocytes, most of which were in a necrobiotic condition. Nodules delimited by 1-2 rows of mononuclear, fat-containing cells were found in individual portions of the tissue. The remainder of the subcutaneous cellular tissue was infiltrated by a large number of mononuclear macrophages, many of which contained an inclusion of fat in the cytoplasm.

Later (after 7 days), the number of oil-phagocytizing, mononuclear cells increased. Due to the more intense resorption, the amount of oil in the subcutaneous cellular tissue decreased. The oil-containing nodules were mostly small and surrounded by 1-2 rows of macrophages, among which a few fibroblasts were found. In other places, single polynuclear cells were seen.

During the later stages of the inflammatory reaction (after 14 days), quite a lot of oil remained in the subcutaneous cellular tissue, contained in nodules of various sizes, contiguous to each other and edged with one row of elongated fibroblast cells (Fig. 1).

The number of mononuclear cells decreased, and only some of them contained an inclusion of oil in the cytoplasm.

When rat fat was injected into the subcutaneous cellular tissue, the reaction was generally the same as when the oil was injected. The difference was that the fat injection caused a greater leukocyte infiltration, with sharply expressed phagocytosis the 3rd day after the injection. Due to the more intense resorption, an appreciably lesser amount of fat remained by the 7th day than remained after the same period with the oil injection (Fig. 2). At later intervals (14 days), one could see many small, loose-lying drops of fat and a small quantity of diffusely scattered cell elements in the subcutaneous cellular tissue.

Morphological Picture in the Animals Injected with the Hypnotic After the Medicamental Sleep Had Passed. When oil was injected into the rat 3 days after the sleep, the main mass of the oil appeared in the form of many large, coalescing drops, surrounded by a small number of polynuclear cells, some in a condition of disintegration. Individual oil drops of various sizes were found lying loosely in the subcutaneous cellular tissue. Mononuclear cells of the polyblast type predominated in the infiltration. However, there were as yet no phagocytes. At later dates (on the 7th and 14th days), the individual oil drops were observed to be delimited by 1-2 rows of mononuclear cells, and the quantity of cell elements gradually decreased; there was a slight increase in phagocytosis, which later ceased completely. Single polynuclear cells were found.

When the oil was injected 7 days after the sleep, the body reaction was somewhat more intense than in the early intervals (3 days). The oil-containing nodules were mostly small, and many of them were surrounded by 2-3 rows of elongated mononuclear cells; the smaller drops lay freely. There were no polynuclear cells. A small number of mononuclear cells were found, some containing oil. Later (on the 7th and 14th days), the proliferation process increased and the oil-containing nodules were surrounded by mononuclear cells of the fibroblast type, arranged in several rows. The number of mononuclear cells in the infiltration increased. However, along with the well-expressed proliferation processes in the central portions of the subcutaneous cellular tissue, on the 14th day we observed even more significant accumulations of polymorphonuclear leukocytes, most of which were in a necrobiotic condition. Phagocytosis was slight.

Fig. 1. Subcutaneous cellular tissue of a control rat on the 4th day after the oil injection. Many oil-containing nodules, separated from each other by narrow layers of elongated cells.

Fig. 2. Subcutaneous cellular tissue of a control rat after the rat fat injection. Massive leukocyte infiltration in the region surrounding the fat-containing nodules.

When the oil was injected 14 days after the sleep had passed, many round nodules of different sizes, surrounded by several rows of mononuclear cells, were observed in the subcutaneous cellular tissue of the rat at early dates (3 days); some of the cells contained drops of oil. There were large as well as small nodules

found. Later (after 7 days), the general quantity of cellular elements decreased slightly. At the same time, the number of cells around the individual nodules increased. Phagocytosis was more marked. The 14th day after the oil injection (i.e.,28 days after the sleep), the majority of the oil-containing nodules were small and situated slightly apart from each other. They were well delimited by 3-4 rows of elongated mononuclear cells, which included many fibroblasts. A large accumulation of lymphoid and histiocytic elements and fibroblasts was observed between the nodules. Individual cells contained inclusions of oil in the cytoplasm (Fig. 3).

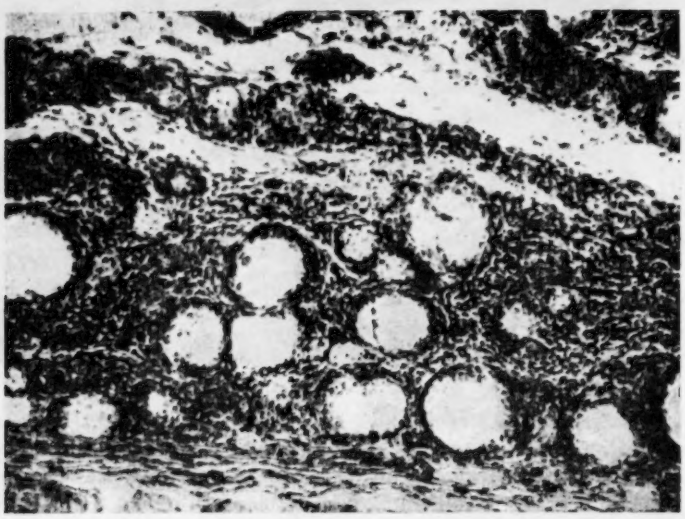


Fig. 3. Subcutaneous cellular tissue of an experimental rat on the 14th day after the oil injection. The injection was done 14 days after the sleep. The oil-containing nodules are well delimited by 3-4 rows of cells, and there is a sharply expressed proliferation reaction between the nodules.

There was a greater change in the reaction to rat fat after the medicamental sleep than in the reaction to oil (Fig. 4). While the injection of fat into the control caused a profuse leukocyte reaction with phagocytosis, in the experimental animals at the early intervals after sleep (3 days), the polymorphonuclear leukocytes were, in general, absent; there was only a small number of mononuclear cells, and phagocytosis was weakly expressed. The 14th day after sleep, the picture approximated that of the control, although there were also rather large nodules, edged with 1-2 rows of elongated fibroblasts, found among the small, fat-containing nodules,which indicated a certain delay in the resorption of the fat.

We have established that the reaction was more intense in the control animals than in the experimental. After the sleep, the intensity of the inflammatory reaction decreased; leukocyte migration was considerably less marked; the leukocyte wall around the individual drops of fat (oil) was absent or small, and phagocytosis was weakly expressed.

One must note that the character as well as the intensity of the inflammatory reaction was different. In the control, the leukocytic character of the reaction was marked, whereas, in the experiment, mononuclear cells predominated from the very first days.

The reactivity of the animals was more markedly changed when the stimulant was injected a short period after the sleep had passed (3 days) and remained changed up to the 7th and 14th days, but to a lesser degree. This was especially true of the later stages of inflammation, induced the 14th day after the medicamental sleep, when the original reactivity had apparently already been restored.

However, even in the very latest periods which we studied, for example the 7th and 14th days after the

oil injection (14 days after the sleep), a difference could be seen, as compared with the control, in the degree of the proliferative reaction, which after the sleep, i. e. under the conditions mentioned above of lowered reactivity, was more significantly expressed.

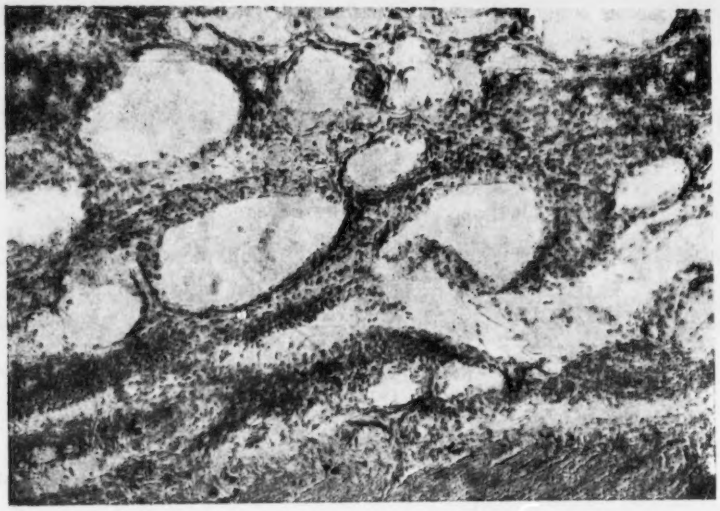


Fig. 4. Subcutaneous cellular tissue of an experimental rat on the 7th day after the rat fat injection. The injection was done 7 days after the sleep. In the region surrounding the fat-containing nodules, there are no polymorphonuclear leukocytes and only a few mononuclear cells.

This extremely interesting phenomenon needs further special study.

The reduced tonicity of the cortex evidently contributes to the different proliferative processes. I. P. Tereshchenko noted this in connection with the intensity of growth in grafted, malignant tumors.

In the laboratory of M. L. Borovsky, N. K. Georgiu [4] observed that the healing processes in the amputated stump of a dog were accelerated by the use of prolonged medicamental sleep.

Thus, from the data given, one can conclude that Barbamyl-induced sleep results in rather prolonged changes in reactivity. At least, such a conclusion follows from the character of the changes which we observed in the inflammatory reaction caused by the stimulants we injected. This reaction lingered, but its character changed toward a decreased intensity of the exudative processes and, inversely, towards the increase of the proliferative phenomena.

The duration of reactivity change can be associated with the duration of sleep, especially since, according to the data of O. Ya. Ostry and A. M. Monaenkov [7], the duration of the medicamental sleep alone can be a characteristic of the initial correlation of the principal nerve processes in the central nervous system, which is connected with this or that condition of reactivity.

It is obvious that, in cases of repeated medicamental sleep therapy, when establishing the dosage, one must take into consideration the after-effect of one-time medicamental sleep which we have mentioned; in this way, the greatest efficacy of the therapy used can probably be attained. This is especially essential in the case of inflammation, when it is necessary to regulate such a basically protective reaction as inflammation so as to eliminate its harmful effect on the organism, which often occurs when this reaction is excessively intense.

SUMMARY

The duration of the effect of medicamental sleep on the character and development of an aseptic inflammation brought about by the subcutaneous injection of fatty substances (peach oil, rat fat) in different intervals after the sleep (3, 7, 14 days) has been studied.

In all the experimental variants the inflammatory reaction lingered, and intensity of the exudative processes decreased, whereas proliferative phenomena increased. Reactivity of the animals was found to be higher if the stimulants were injected shortly after the sleep. In the latest intervals under observation (14 days) a difference in the morphological picture could nevertheless be observed in comparison to the control animals.

LITERATURE CITED

[1] R. S. Braude and S. M. Gitman, in the book: Collection of the Works of the Central Skin Venereology Institute,* Moscow, 1941, Vol. 8, pp. 233-240.

[2] M. L. Biryukov, in the book: Abstracts of the Reports of the Second All-Union Congress on Problems of Industrial Toxicology,* Moscow, 1952, pp. 8-8.

[3] I. P. Vinogradov, Vestnik Khirurgy imeni Grekova, 1935, Vol. 99, books 110-111, pp. 52-60.

[4] N. K. Georgiu, Experimental Morphological Analysis of the Pathological Process Developing Due to the Amputation of a Dog's Extremity.* Report of Works Conducted, Dissertation, Moscow, 1953.

[5] K. F. Dogaeva, and S. I. Itkin, in the book: Problems of Clinical and Experimental Surgery,* Moscow, 1951, pp. 60-66.

[6] V. V. Lauera, Vrachebnoe Delo, 1927, No. 4, pp. 261-264.

[7] O. Ya. Ostry and A. M. Monaenkov, Byulleten Eksperimentalnoi Biology i Meditsiny, 1955, No. 5, pp. 40-42.

[8] T. A. Pisareva, Nov. Arkhiv Khirugiya, 1930, Vol. 20, book 1, pp. 97-103.

[9] N. M. Rapoport, in the book: Authors' Reports of the Scientific Meeting of the Saratov State Medicine Institute,* Saratov, 1952, p. 142.

[10] A. D. Troitskaya, in the book: Pyodermites,* Moscow-Leningrad, 1953, pp. 32-40.

[11] I. Ya. Uchitel, in the book: Abstracts of the Reports from the Joint Meeting of the Scientific Council of the Institute of Physiology, Acad. Med. Sci., USSR, with the Scientific Councils of the Institutes of Pharmacology, Neurology, Surgery, Pediatrics and Experimental Medicine, Acad. Med. Sci., USSR to Discuss Experimental Problems Concerning Sleep Therapy* (October 12-15, 1954), Moscow, 1954, pp. 56-57.

* In Russian.

CHANGES IN THE PERIPHERAL NERVOUS SYSTEM DUE TO EXPERIMENTAL FEVER

V. V. Astakhova

From the Department of Normal Anatomy (Head – Prof. B. A. Dolgo-Saburov, Corresponding Member of the Academy of Medical Sciences, USSR), the S. M. Kirov Military Medical Academy, and the Department of General Pathology (Head – Prof. P. N. Veselkin, Corresponding Member of the Academy of Medical Sciences, USSR), the Institute of Experimental Medicine of the Academy of Medical Sciences, USSR, Leningrad

(Received January 28, 1956. Presented by V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR)

Studying the condition of the peripheral nervous system during certain diseases, B. I. Lavrentyev and his colleagues [13, 14] found a series of changes in the nerve fibers and in their endings which were not characteristic of Wallerian degeneration. They were mainly expressed in the disturbance of the ability to adsorb silver salts, in surplus growth and in the formation of varicosities, which sometimes were like coarse thickenings deforming the fiber. The earliest changes, which are reversible, B. I. Lavrentyev treated as "symptoms of irritation". Such an affection of the nerve apparatuses, prior to change in the innervated tissue, he considered to be the morphological equivalent of disfunction of the nerve fiber and synapse.

The studies of many authors have shown that reaction changes of the peripheral nerve apparatuses are observed in the most diverse diseases, when, as under experimental conditions, they are first detected in the afferent part of the peripheral nervous system.

The purpose of our study was to investigate the presence and character of morphological changes in certain elements of the peripheral nervous system under fever conditions.

The works of P. N. Veselkin [1-3] and his colleagues have established the importance of the nerve mechanisms, and that of the peripheral neuro-receptoral apparatuses in particular, to the realization of a fever reaction.

B. A. Dolgo-Saburov [4, 5, 6, 7] and V. V. Kupriyanov [10, 11] have shown the high reactivity of the receptoral apparatuses in the wall of the caval veins and vessels of the lesser circulatory system during different pathological conditions (de-efferentation, oxygen deficiency, uremia, anemia, etc.). We conducted our studies on these same vessels and on the portal vein. Besides the receptor fibers and their endings, we also studied the nerve trunks (vagus nerves) and the cell bodies of the neurons which provide sensory innervation of the indicated veins, i. e., we studied the nodose ganglia of the vagus nerves and the intervertebral ganglia of the corresponding segments of the spinal cord.

EXPERIMENTAL METHODS

Cats were used as the experimental animals. The fever reaction was caused by a subcutaneous hip injection of a Bacillus mesentericus culture. In one series of experiments, 2 ml of a pyrogenic culture to each kilogram of the animal's weight (optimal dose) was injected, in the other – 8 ml for increased irritation.

The experiments were done on 50 cats in series of five animals each. The cats were kept in identical conditions for 2-3 days, then three of them were injected with the pyrogenic irritant, and the two control animals were injected with the same amount of a physiological solution.

The temperatures – the original and that after the irritant injection – showed a fever reaction in all of the animals. The maximal temperature increase was 2°. The larger doses of the injected irritant caused the same temperature reaction.

The animals were killed by ether inhalation.

Material for morphological examination was taken after 1, 3, 4, 6-8, 15-18 and 24 hours, and also 6 days after the beginning of the experiment.

The items to be studied were histologically processed according to the methods of Bielschowsky–Gross, Ranson, Nissl and Marchi.

EXPERIMENTAL RESULTS

Examination of the walls of veins taken from the animals one hour after the pyrogenic irritant injection showed no change in the condition of the peripheral nerve apparatuses.

A study of preparations obtained 3-4 hours after the culture injection, i. e., the beginning of the increased temperature period (in the injections of both the optimal and larger doses of the pyrogenic irritant), showed that reaction changes were present in the nerve apparatuses in the caval and pulmonary vein walls. The same was noted on preparations obtained 5-8 hours after the culture injection, i. e., the period of maximal temperature rise.

Along with a heightened affinity to silver (argentophilia), considerable thickening of the myelinated nerve fibers was noticed, mainly in their preterminal sections. Some of the swollen, deformed fibers ended in a dense terminal branching (Fig. 1). In others, we also noticed a marked coarsening and thickening of the actual endings, the branches of which were greatly impregnated and looked like large bulbs. In the vein walls of animals killed 3-4 hours after the injection, we often found such nerve fibers which terminated in a blunt, "stump-like" end among a mass of special cells forming a "sensory bed" (Fig. 2).

Secondary Wallerian- type degeneration of the fibers was, as a rule, not observed. Unchanged nerve endings appeared relatively rarely on the preparations obtained 3-4 hours after the culture injection. There were no deviations from normal structure in the nervous apparatuses of the portal vein wall.

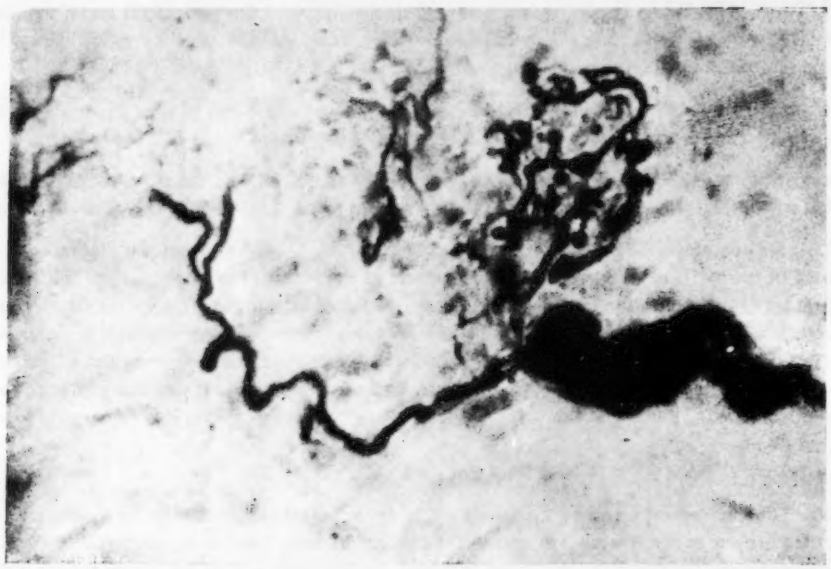


Fig. 1. Coarse thickening in the preterminal section of a receptor fiber in the wall of the superior vena cava of a cat.

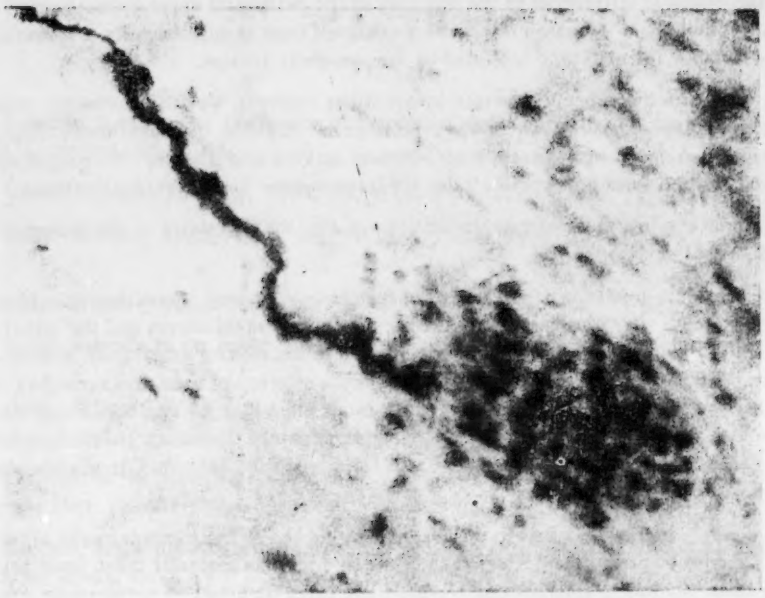


Fig. 2. "Stump" of a nerve fiber, surrounded by a mass of special cells in the wall of the pulmonary vein of a cat.

Different results were obtained from studying vein preparations made from animals killed at later intervals after the pyrogenic substance injection. 15-18 hours after the start of the experiment, i. e., during the post-fever period, a larger number of normal sensory fibers and their endings was observed, and changed nerve apparatuses were found much less frequently. Among these, appeared receptors in the form of thin, frequently palely-impregnated, sparse branchings coming off the end or side of the fiber (Fig. 3). Sometimes similar branchings sprouted directly from the spherical thickening of the preterminal section of the fiber.

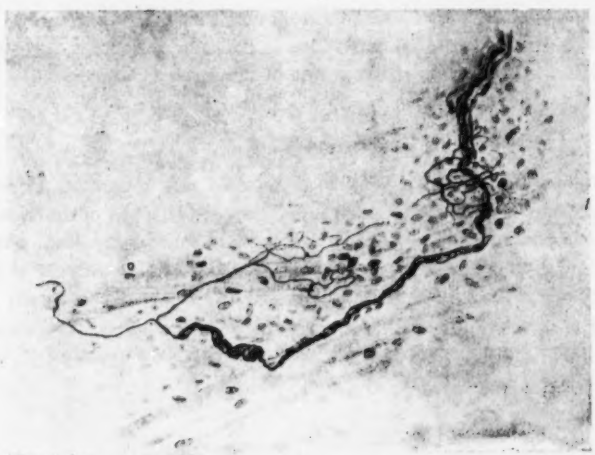


Fig. 3. Thin, delicate branchings on the end and side of a myelinated fiber in the wall of the superior vena cava of a cat.

Studying the vein preparations obtained still later after the start of the experiment – after 24 hours, in general, we did not find essential differences in the sturcture of the peripheral nerve apparatuses of either the experimental or the control animals. The same results were obtained from examining vein preparations from cats which had lived 6 days after the one-time injection of the pyrogenic irritant.

Studying the veins which had been processed by the silver methods, we did not observe changes in the elements of their efferent innervation in any of the experiments. The thin, nonmyelinated fibers of the vegetative nervous system were evenly impregnated, their contours uniform and smooth. The ganglionic nerve cells in the vein wall were normal; sometimes pericellular apparatuses were clearly evident on them.

These observations confirm the long-established opinion that the reactivity of the sensory innervation circuits is greater than that of the motor.

In order to examine the condition of the bodies of the sensory neurons, whose dendrites form the wall receptors of the vessels studied, we examined the nodose ganglia of the vagus nerves and the spinal ganglia of the upper and middle thoracic segments of the spinal cord, which are the sensory innervation sources of the caval and pulmonary veins. We noted only single cells with symptoms of primary irritation according to Nissl, or, more rarely, with serious changes. By computation, it was established that the number of such cells in the preparations from the experimental and control animals was approximately the same. No changes were found on longitudinal and lateral sections of the vagus trunks which were processed according to the Marchi method to determine the reaction of the afferent fibers.

The reaction changes that we did find in the receptor fibers and in their endings were all of the same type. It is true that some features of these changes were more usually found on material taken from animals at earlier intervals (the nerve fiber "stumps"), and that others were more often observed on preparations obtained from cats which had lived longer after the start of the experiment (the thin, pale terminal branches, which we believe to be a young form of receptor).

One can suggest that these and other features of the changes are the morphological expression of the different functional conditions of the neuron.

The nerve fiber "stumps" found on the histological preparations allow the proposal that autoreceptorotomy (similar to N. I. Zazybin's autoneurotomy) is possible as an adapting reaction of the neuron in response to the action of the injuring factor, with the subsequent regeneration of that neuron's dendrite and thus the restoration of the receptor. The great capacity of nerve fibers for growth and the considerable variability of the peripheral nerve apparatuses have been repeatedly mentioned by K. A. Lavrov [12] and by N. I. Zazybin [8, 9]. Regeneration of the receptors in the wall of the estuarial sections of the caval veins after their destruction, with a disturbance of the portal circulation, was observed by B. A. Dolgo-Saburov [7] in cats.

The character and sequence of development of the reaction changes give reason to suggest that they are morphological expressions of different functional conditions of the sensory neurons, as of the initial circuit of the analyzor mechanism, which first experiences the action of the foreign factor.

SUMMARY

Studies of the nervous apparatus of the large veins in animals with experimental fever caused by the injection of a culture of Bacillus mesentericus demonstrated reactive changes of the sensory apparatus in the walls of the superior vena cava and pulmonary veins with intact afferent innervation. Structural changes mainly in preterminal and terminal sections of the medullated fibers were as a rule detected in 3 hours after the injection of the stimulus, reached the maximum in 6-8 hours and disappeared in 24 hours. Neurons of the afferent innervation in walls of the g. nodosa and the spinal ganglions were not changed. The data obtained show that reactive changes caused by experimental fever first appear in the peripheral section of the neuron and are reversible.

LITERATURE CITED

[1] P. N. Veselkin, Fiziologichesky Zhurnal SSSR, 1939, Vol. 26, No. 6, pp. 672-686.

[2] P. N. Veselkin, in the book: Collection of Scientific Works Dedicated to the 60th Birthday of the Academician Orbeli,* Leningrad, pp. 45-54.

[3] P. N. Veselkin, Arkhiv Patology, 1952, No. 4, pp. 3-20.

[4] B. A. Dolgo-Saburov, Byull. Eksptl. Biol. i Med., 1949, Vol. 28, No. 8, pp. 146-151.

[5] B. A. Dolgo-Saburov, Vrachebnoe Delo, 1950, No. 10, pp. 903-910.

[6] B. A. Dolgo-Saburov, in the book: Nervous Regulation of Circulation and Respiration,* pp. 241-247, Moscow 1952.

[7] B. A. Dolgo-Saburov, in the book: Abstracts of the Reports of the All-Union Congress of Pathologico-Anatomists,* Leningrad, 1954, pp. 3-5.

[8] N. I. Zazybin, in the book: Works of the 5th All-Union Congress of Anatomists, Histologists and Embryologists,* Leningrad, 1951, pp. 597-602.

[9] N. I. Zazybin, in the book: Abstracts of the Reports of the All-Union Congress on Neuromorphology,* Leningrad, 1952, pp. 15-16.

[10] V. V. Kupriyanov, Byull. Eksptl. Biol. i Med., 1950, Vol. 20, No. 10, pp. 291-295.

[11] V. V. Kupriyanov, The Innervation of the Vessels of the Lesser Circulatory System,* Dissertation, Leningrad 1953.

[12] K. A. Lavrov, Terminal Sections of the Peripheral Nervous System,* Rostov-on-Don, 1941.

[13] A. G. Filatova and B. I. Lavrentyev, Byull. All-Union Eksptl. i Med., 1934, No. 10, pp. 11-13.

[14] B. I. Lavrentyev and I. M. Lasowsky, Ztschr. f. d. ges. Neurolog. u. Psychiatr., 1931, Bd. 131, H. 4/5, S. 585-601.

* In Russian.

THE CONDITION OF THE VASCULAR-CAPILLARY SYSTEM OF THE BRAIN IN ANIMALS OF DIFFERENT AGES DURING ASPHYXIA

Article II

T. P. Zhukova

From the Laboratory for the Study of Brain Development (Director: Prof. B. N. Klosovsky, Corresponding Member of the Academy of Medical Sciences, USSR), the Institute of Pediatrics (Director: Prof. O. D. Sokolova-Ponomareva), Academy of Medical Sciences, USSR, Moscow

(Received March 5, 1955. Presented by Prof. G. N. Speransky, Active Member of the Academy of Medical Sciences, USSR)

This work is part of a research project studying the effects of different physiological and pathological body conditions on the blood supply and the vascular-capillary system of the cerebral hemispheres in animals of different ages.

The preceding article [2] presented data regarding change in the blood supply of the cerebral hemispheres due to asphyxia in cats of various ages. By quantitatively determining the amount of blood supplying the brain, we showed that the blood supply to the cerebral hemispheres increased after lethal asphyxiation in kittens 1-2 days old, 25-45 days and 2-3 months old, and in adult cats as well. Only in kittens which had died from asphyxia when 3-10 days old was there no change in the blood supply to the cerebral hemispheres. In this age group, the amount of blood going to the cerebral hemispheres increased at the start of asphyxia, but after the death of the animal, the amount of blood supplied to the cerebral hemispheres was reduced to normal.

EXPERIMENTAL METHODS

The animals were asphyxiated by being put into a hermetically sealed chamber twice the volume of the animal. As soon as respiration ceased, the animals were withdrawn from the chamber and decapitated. The skull was opened and the brain removed; the right hemisphere was isolated – it was used for the quantitative blood determination; the left hemisphere and the rest of the brain were placed in 7-10% formalin for subsequent histological processing. Sections from the frontal-parietal and occipital regions of the cerebral hemispheres were used for the histological study. The brains of healthy animals of the same age were processed concurrently by the same method as those of the animals which had been asphyxiated.

By histologically processing the brains of these animals and using one of the cerebral hemispheres for quantitative blood determination, we could compare data concerning the quantitative blood determination with that concerning the morphological condition of the vascular-capillary system of the brain from the same animal.

The sections were histologically processed by the Eros method, which stains erythrocytes with acid fuchsin, by the Masson method, which stains both plasma (with aniline blue) and erythrocytes (with acid fuchsin), and by the Klosovsky method, which is based on silver impreganation of the vascular wall. To stain preparations by the Eros method, paraffin blocks were cut into sections 60 microns thick. The sections stained according to Masson were 7 and 20 microns thick. Sections 150 microns thick were made from celloidin blocks of the brain, processed by the Klosovsky method.

EXPERIMENTAL RESULTS

On brain preparations processed by the Eros method from normal kittens, a weak acid fuchsin coloring of the hyaloplasm was noted; in some places, the nerve cells were also stained. A small number of capillaries containing eythrocytes, which were arranged in the form of a few sparse chains (Fig. 1) were observed in the cortex and white matter. A somewhat different picture was seen in the subcortex. Here, there were considerably more blood-filled capillaries than in the cortex of the cerebral hemispheres. It is a known fact that the subcortex is more mature than the cortex of the cerebral hemispheres in young animals born prematurely. This could explain the greater vascularization of the subcortex as compared with the cortex of the cerebral hemispheres.

Fig. 1. Vascular-capillary system of the cerebral hemisphere cortex in the brain of a one-day old healthy kitten. Eros method of staining. Magnification 15 × 10.

Studying the preparations processed by Masson's method, we noted that a large part of the capillaries of the cortex and white matter were filled with plasma and contained few erythrocytes. The fact that a relatively small number of blood-filled capillaries was observed on the preparations stained according to the Eros method (which only shows erythrocytes) is explained by the large quantity of plasma present in the capillaries and in some of the larger vessels.

On the preparations impregnated with silver according to the Klosovsky method, many constricted capillaries were evident in the cortex, and a lesser number in the white matter. Many of the capillary lumina were not uniform in diameter. The tonicity of the vascular wall was poor, causing the capillary network forming the vascular-capillary system of the cortex to be misshapen.

The same condition of the vascular-capillary system of the cerebral hemispheres in kittens a few days old was also noted by Z. N. Kiseleva [3], who showed that the brains of healthy kittens are edematous during the first few days of life.

In the previous article, we showed that the amount of blood in the cerebral hemispheres of normal kittens 3-10 days old is slightly greater than that in 1-2 day old kittens; the cerebral hemispheres of normal kittens 1-2 days old contain an average of 0.81% blood, while those of normal kittens 3-10 days old contain an average of 0.88% blood. Such a slight change in the cerebral hemisphere blood supply is almost imperceptible on histological preparations. Therefore, the condition of the cerebral hemisphere vascular-capillary system in normal 3-10 day old kittens is practically the same as that in normal kittens 1-2 days old. However, one must note that, after the 3rd day of life, an increase in the number of growing capillaries was observed.

We have established that the blood supply to the cerebral hemispheres of animals increases as the body of the animal grows and develops. Only in animals aged from 1½-2 months does it approximate the amount characteristic to the mature body. The morphological condition of the vascular-capillary system of the cerebral hemispheres also changes accordingly. Studying histological preparations made from the vascular-capillary system of the brain in normal kittens 20-45 days old, we observed an increased amount of blood in the vessels of the cerebral hemispheres, a more dense distribution of eythrocytes in the capillaries, and some increase in the lumina as compared with the capillaries of normal 10 day old kittens. At the same time, some differences in the condition of the vascular-capillary system of the cerebral hemispheres could also be observed in this age group. In 20 day old kittens, for example, there were fewer capillaries manifested by the Eros method and fewer with irregular lumina than were found in 30 day old kittens, etc. As the age of the kitten increased, the hyaloplasm stained less and less with acid fuchsin. Therefore, vessels stained with acid fuchsin were visible on a light background in normal kittens more than 30 days old and in mature animals. The decreased intensity of the acid fuchsin stain in the hyaloplasm indicates the reduced acidity of the basic brain substance, which, in turn, is connected with the gradual disappearance of edema in the brain as the kitten grows older. The condition of the vascular-capillary system of the cerebral hemispheres in normal kittens 1.5-2 months old was almost identical with that of the mature body. At this age, however, growing capillaries were still rather frequently found in individual portions of the brain [1].

The picture of the condition of the cerebral hemisphere vascular-capillary system was different in the animals of various ages which had died from asphyxia.

In 1-2 day old, asphyxiated kittens, marked dilatation was observed in the veins and arteries of the cortex (Fig. 2), white matter, subcortex, and in the vessels of the pia mater of the brain; erythrocyte-containing capillaries were considerably more numerous than in the brains of normal kittens of the same age. There were also much fewer of the capillaries filled with plasma alone. Moreover, in this age group of asphyxiated kittens, capillaries with uniform lumina predominated, and a slight dilatation of the capillaries was also observed.

Fig. 2. Vascular-capillary system of the cerebral cortex of a one-day old, asphyxiated kitten. Eros method of staining. Magnification 15 x 10.

Histological study of the cerebral hemispheres from 1-2 day old, asphyxiated kittens confirmed the information obtained from quantitative blood determination, i. e., that the blood supply increased. Analyzing the preparations made from the cerebral hemispheres of these kittens, we observed also that the larger vessels, particularly, were dilated and that the capillaries were more filled with blood because of the increased blood supply to the cerebral hemispheres (Fig. 2).

As we have already mentioned, compared with the normal animals we found no changes in the blood supply to the cerebral hemispheres of 3-10-day old, asphyxiated kittens. However, histological examination disclosed some peculiarities in the condition of the vascular-capillary system of the cerebral hemispheres from the kittens which had been asphyxiated. On the preparations processed by the Eros method, an intense acid fuchsin coloring was often noticed in the hyaloplasm of the cortex and sometimes in the nerve cells. The vascular walls and the plasma often were stained as well as the erythrocytes. In the cortex and white matter, a relatively small number of erythrocyte-containing capillaries was observed. Finally, in those cases where we discovered capillaries especially poorly filled with blood in the cortex, the dilatation of individual venous vessels was observed in the white matter of the brain.

The preparations processed by the Masson method showed a large number of plasma-filled cortical

capillaries and individual vessels which contained mostly plasma. The stronger acid fuchsin coloring of the hyaloplasm, nerve cells, vascular walls and plasma indicated that the brain tissue of the asphyxiated animals had a more acid reaction to the medium than the brain tissue of normal animals. All the brain tissure properties enumerated above indicate an expressed condition of brain edema [4].

It should also be mentioned that a large number of constricted capillaries with uniform lumina was found on the cerebral hemisphere preparations impregnated with silver. There were less capillaries with irregular lumina than normally.

Therefore, although quantitative blood determination in the greater cerebral hemispheres of asphyxiated kittens 3-10 days old could not show results in any way differing from those received upon analogous examination of healthy kittens of the same age, histological examination showed that there was a difference which consisted in the presence of more sharply expressed symptoms of edema in the brains of the asphyxiated kittens.

Fig. 3. Vascular-capillary system of the cerebral hemisphere cortex of a mature cat after asphyxiation. Eros method of staining. Magnification 15 × 10.

The data presented concerns 3-10 day old kittens, used in the experiment 15-30 minutes after eating, i. e., in the condition we assumed to be normal. If the kittens had been experimented on 2-6 hours after eating, there would have been an increase of the blood supply to the cerebral hemispheres under conditions of asphyxia. Histological examination of the cerebral hemispheres of such kittens showed practically the same features of the vascular-capillary system as were present in kittens 1-2 days old after lethal asphyxia.

In kittens 20 days old or older which had died from asphyxia, most of the capillaries were dilated and well-filled with blood in all sections of the cerebral hemispheres. In contrast to the kittens only a few days old, constricted capillaries and capillaries with irregular lumina were rarely found in this age group.

The large arterial and venous vessels were also dilated in all sections of the cerebral hemispheres.

In 30-45 day old kittens, capillary dilatation was more marked than in the 20 day old kittens.

Practically speaking, the condition of the vascular-capillary system of the cerebral hemispheres in such kittens was almost the same as that in mature cats which died from asphyxiation (Fig. 3).

On silver-impregnated brain sections from mature cats, one could see short, sharply constricted capillaries which looked like threads 1-1.5 microns in diameter, although there was a predominance of dilated capillaries.

Therefore, in kittens of the older age groups and in mature cats, the increased blood supply to the cerebral hemispheres after lethal asphyxia results in the dilatation of all the components of the vascular system.

The following is important in comparing the condition of the vascular-capillary system of the cerebral hemispheres in animals of different ages which have died from asphyxia. In kittens up to 10 days old (especially in 1-2 day old kittens and in hungry kittens 3-10 days old), a sharp dilatation of the larger vessels and, to a lesser degree, of the capillaries was noticed. Dilatation of the large vessels was more pronounced in kittens of this age group than in older kittens and mature cats. Here, it must be taken into account that the brain is somewhat smaller in kittens a few days old than in mature animals. At the same time, the diameters of the large vessels of the cerebral hemispheres in kittens 1-2 days old and in hungry kittens 3-10 days old, which have died from asphyxia, is either the same or greater than that in mature animals. Moreover, the older the animal, the more blood-filled the capillaries and the wider their lumina.

Consequently, histological examination of the cerebral hemispheres from cats of different ages which died from asphyxia confirms the data obtained from quantitative blood determination which was presented in the first article.

SUMMARY

Some peculiarities in the reactions of the vascular system of the brain in one-month old kittens were noted with the aid of the histologic method. Dilatations of large vessels and dilatations to a lesser degree of capillaries has been noted during asphyxia in newborn kittens, likewise in 3-10 day old hungary kittens. 3-10 day old kittens (which had been previously fed) died of asphyxia with a pronounced edema of the brain. The reaction of the cerebral vessels at the end of the first month of life in cases of asphyxia is practically the same as in a mature animal.

LITERATURE CITED

[1] E. G. Balashova, On the Problem of the Correlation of Nerve Cells and Capillaries in Certain Areas of the Brain,* Dissertation, Moscow, 1953.

[2] T. P. Zhukova, Byulleten Eksperimentalnoi Biology i Meditsiny, 1955, Vol. 40, No. 12, pp. 27-32.

[3] Z. N. Kiseleva, in the book: Academy of Medical Sciences, USSR, Reports on Scientific Research,* 1948, No. 5, pp. 36-38.

[4] B. N. Klosovsky, in the book: Works of the N.N. Budrenko, Academician, Institute of Neurosurgery,* Moscow, 1948, Vol. 1, pp. 21-44.

* In Russian.

CHANGES IN THE ORGANS OF WHITE RATS DUE TO AN INTRAPERITONEAL INJECTION OF QUARTZ DUST

G. V. Belobragina

From the Laboratory of Toxicology (Director: Prof. A. L. Yudeles), Sverdlovsk Institute of Labor Hygiene and Occupational Pathology (Director: V. A. Mikhailov, Biological Sciences candidate)
Scientific Directors of the work: Prof. P. P. Dvizhkov and Prof. A. L. Yudeles

(Received February 27, 1956. Presented by A. A. Letavet, Active Member of the Academy of Medical Sciences, USSR)

The literature does not contain much research studying the effect of dust containing different concentrations of silicon dioxide injected into the peritoneal cavity of experimental animals [3, 4].

Therefore, we decided to study the changes which occur in the internal organs of white rats due to an intraperitoneal injection of quartz dust.

EXPERIMENTAL METHODS

The experiments were done on 8 white rats, which were injected once intraperitoneally with 100 mg of quartz dust containing 98.6% free silicon dioxide. Eighty-nine per cent of the dust we used consisted of particles 1 micron or smaller in size, the remainder, of particles 2 microns or smaller. All the animals received the dust injection without visible discomfort, and their condition was completely satisfactory throughout the experiment. Four of the rats were killed after 68 days, and the other four were killed 5 months after the dust injection.

EXPERIMENTAL RESULTS

In the peritoneal cavity of the rats killed 68 days after the dust injection, we observed many, round, dense grayish formations varying in size from those scarcely visible to the naked eye to those the size of a small pea, which were distributed on the parietal peritoneum, in the epiploon, the mesentery, and the intestinal serous membrane. When the largest formations were opened, the injected dust was found in their central portions.

In the rats which had been killed 5 months after the dust injection, the number of the formations described above in the peritoneum was much greater – they studded the peritoneum, the mesentery and the epiploon.

The nodular structure of these formations in all the animals was established by microscopic examination. They seemed identical to the silicotic nodes which form in the lungs of animals after an intratracheal injection of quartz dust. Histologically, they were round, miliary or larger, nodules (Fig. 1), sometimes too large to fit the field of vision in a small microscope magnification. They were rarely situated apart from each other; most of them were in conglomerations, distributed between the individual intestinal loops, causing the loops to coalesce with each other or with the peritoneal wall. The cellular composition of the nodules and nodes was analogous to that observed in the lungs after an intratracheal quartz dust injection: histiocytes, cells of the epithelial type, and lymphocytes – the lymphocytes were usually distributed over the surface of the nodules. Many silicon dioxide particles were observed in the histiocytes and in the epithelial-type cells. Silvering the 68 day sections according to Foot showed that the framework of nodules consisted of a thick net of concentrically arranged, collagenous fibers; the number of fibers gradually decreased towards the center of the nodules.

The central portion of many of the nodules stained yellow with picric accid, and did not have a definite morphological structure – only loose dust and cell detritus were found in it, there were no fibers or cells. Newly-formed, thin-walled, blood capillaries could be seen on the surface of the nodules.

In the animals killed 5 months after the quartz dust injection, the number of collagenous fibers on the nodular surfaces was much greater – many were discovered in the central portions also, but the node itself remained structureless and contained only dust. When the sections were silvered, a thick, developed net of argentophilic fibers was discovered in the nodules.

At the same time, we observed hyperplastic retroperitoneal, para-aortal, and portal lymph nodes in the animals. When the lymph nodes were examined histologically, dilatation of the lymph sinuses with an increased number of dust-containing reticuloendothelial cells and the presence of nodular formations consisting of reticuloendothelial, proliferative cells were observed. The reaction in the above lymph nodes to the intraperitoneal injection of quartz dust was so strongly expressed that only separate islands of lymphoid tissue remained in them, while the main mass of the node was represented by multiplying reticuloendothelial cells, containing dust particles in their cytoplasm (Fig. 2).

The lymph nodes of the rats which had been killed after 5 months showed delicate collagenous fibers in the marginal sinuses.

In the liver of rats killed 68 days after the dust injection, there were many foci of submiliary size (Fig. 3) consisting of reticuloendothelial elements distributed in the periportal tissue, between the hepatic cords, with a developed net of argentophilic and collagenous fibers. We also observed that the nuclei of the hepatic cells were of different sizes.

In the spleen, both 68 days and 5 months after the injection, a different number of miliary foci was found, consisting of reticuloendothelial elements and primarily distributed in the center of the follicules. In the pulp, there was a large quantity of dark brown pigment which did not give a positive reaction to iron (quartz dust) and also a pigment which did give a positive reaction to iron (hemosiderin).

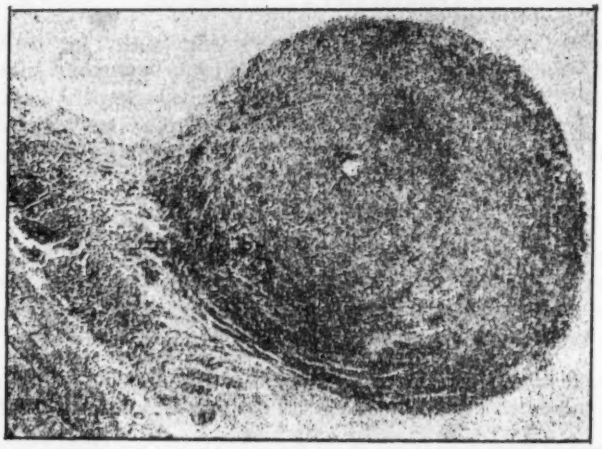


Fig. 1. Silicotic nodule on the parietal peritoneum of a rat killed 68 days after an intraperitoneal injection of 100 mg of quartz dust. Ocular 5, objective 10.

After 68 days, according to the dust deposit and to the development of granulation tissue on the serous membrane of the small intestine, the villi on the mucous membrane of the small intestine were observed to decrease in number and size and to be sclerosed. The mucous membrane situated directly above the granulation tissue portions lost almost all its villi. After 5 months, the changes of the mucous membrane were characterized by more marked focal areas of atrophy.

Fig. 2. Retroperitoneal lymph node of a rat killed 68 days after an intraperitoneal injection of 100 mg of quartz dust. Small focal nodular proliferation of the reticuloendithelial elements. Ocular 7, objective 10.

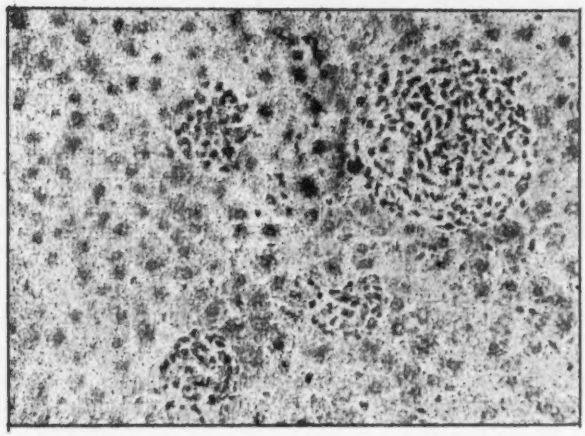


Fig. 3. Submiliary and miliary foci from the proliferating reticuloendithelial cells in the liver of a rat killed 5 months after an intraperitoneal injection of 100 mg of quartz dust. Ocular 7, objective 40.

Single foci of small, round cell and histiocyte infiltration were found between the convoluted tubules in the kidneys.

In the lungs, we found histiocyte multiplication in the interalveolar septi, which led to a considerable thickening of the septi, and lymphoid infiltration was noted around the small vessels and bronchi. The peribronchial lymph nodes were slightly hyperplastic, and a small accumulation of large cells with foamy protoplasm was found in them. There were no typical silicotic nodes in the lungs and regional lymph nodes in the animals of this series.

There were no changes in the other organs examined.

The studies which have been done show that typical silicotic nodes usually form according to the place at which the animal is injected with quartz dust. If the dust is injected intratracheally, the nodes will appear in the lungs and the regional (bifurcated) lymph nodes; if the dust is injected into the peritoneal cavity, the nodes will appear in the epiploon, along the serous membrane of the peritoneal cavity and in the regional lymph nodes. When the nodes appearing in the lungs and those appearing in the peritoneal cavity are structurally compared, it is evident that their cellular composition is identical and that they originate from the same cellular elements, which belong to the reticuloendithelial system (N. N. Anichkov).

The changes in the liver and spleen, i. e., the small foci forming from multiplying cells of the reticuloendothelial type, with the subsequent formation of argentophilic and collagenous fibers in these foci, belong to this category of changes. In the lungs, however, we observed no nodular formations to develop.

These studies also show that the intraperitoneal method of dust injection can be used as an index to test the silicogenic action of different sorts of dust.

SUMMARY

Intraperitoneal injection of 100 mg of quartz dust to white rats led to the appearance of typical silicotic nodes in the peritoneum, epiploon, mesentery and intestinal serous membrane. Simultaneously in the regional lymph nodes there developed focal and diffuse reticuloendothelial elements and later there also appeared collagenous fibers. In the liver and spleen there were small foci of developing reticuloendothelial elements with the appearance of the argentophilic and collagenous fibers. In the intestinal tissue of the kidneys, infiltrates developed and atrophy appeared in the mucous membrane of the intestine. No typical silicotic nodes developed in the lungs, but an infiltrative and proliferative process was observed.

LITERATURE CITED

[1] P. P. Dvizhkov, Gigiena i Sanitariya, 1954, No. 9, pp. 26-32.

[2] P. P. Dvizhkov, in the book: Combatting Silicosis* Moscow, 1953, pp. 244-283.

[3] C. Legerlotz, Beitrage zur Silikose-Forschung, 1955, H. 36, Bochum.

[4] L. Sielvermann and A. R. Moritz, Arch. of Ind. Hyg. a. Occup. Med., 1950, Vol. 1, No. 5, pp. 499-505.

* In Russian.

NEW METHODS

METHOD FOR THE HISTOMETRIC DETERMINATION OF POTASSIUM

M. G. Shubich

From the Chair of Histology (Head – Assistant Prof. G. F. Berezentseva)
of the Kuban Medical Institute, Krasnodar (Director – Prof. V. K. Suprunov)

(Received September 28, 1956. Presented by Full Member of the Acad. Med. Sci. USSR, V. N. Chernigovsky)

Despite the importance of histological determination of potassium, the possible methods for this are very limited. The Macallum method [6] (precipitation of potassium by sodium hexanitrocobalt in the form of $K_2Na[Co(NO_2)_6]$) and conversion of this compound into cobalt sulfide, which can be detected microscopically, has been doubted by many authors [1, 3, 4]. According to Lison [5] the Macallum technique is of no value in histochemical studies of potassium location.

The Carere-Comes method [4], based on the formation of an orange-red precipitate by sodium dipicrylamine with potassium, has a number of advantages: the colored product is formed by direct reaction between sodium paradipicrylamine with potassium, because of the rapid penetration of the reagent into the tissue the colored product is formed at the site of location of the potassium, the preparation technique is relatively simple, and the preparations themselves are stable and do not fade. However, the specificity of this reagent in histological applications gives rise to doubt. Sodium paradipicrylamine has an orange-red color, and the color of the final product, potassium paradipicrylamine, which serves to show the location of potassium in the tissues, is the same. It is therefore possible that, in addition to the formation of potassium paradipicrylamine, simple staining of one microstructure or another by sodium paradipicrylamine may occur.

In such cases control experiments are of fundamental importance.

It must be pointed out that Carere-Comes did not give either the method for preparing the reagent or the concentrations of the solutions in his paper. Aqueous sodium paradipicrylamine solution was patented by him under the name of "Carere-Comes Sienna Orange," and is distributed by one foreign firm. This makes the use of the Carere-Comes method very difficult, and is probably the reason why our national literature contains no reports of its use.

The usual method for preparing paradipicrylamine by nitration of diphenylamine requires a well-equipped chemical laboratory; we obtained a negligible yield because of resinification of the reaction mass. A more convenient method used by us, is preparation of sodium paradipicrylamine from an available product – ammonium dipicrylamine (aurantia dye). A saturated solution of aurantia is made in ethyl alcohol, decanted, and 10% sulfuric acid is added to it until precipitation ceases. The precipitate is filtered off under suction, transferred to a flask, and excess sodium carbonate is added to it. The mass is boiled for an hour on the water bath and filtered. The solution can be used for the experiments. 1 1/2 to 2-fold dilution is permissible.

To ensure the specific nature of the histochemical reaction for potassium with sodium paradipicrylamine, a control technique was developed. The reagent for treatment of the control preparations is phospho-12-tungstic acid which, as was shown by Nikitina [2] precipitates potassium according to the equation:

$$5K^+ + 2H_3(PO_4 \cdot 12WO_3) \rightarrow K_5H(PO_4 \cdot 12WO_3)_2 \cdot 6H_2O + 5H^+.$$

When a preparation is treated with phospho-12-tungstic acid all the potassium present in the section is converted into potassium phospho-12-tungstate, and does not react with sodium paradipicrylamine. Simple staining of microstructures by sodium paradipicrylamine, if it occurs, is not specifically influenced by treatment with phospho-12-tungstic acid.

The control experiments showed that the orange-red, orange-brown, orange, orange-yellow, and clear yellow colors of microstructures treated by the Carere-Comes method are the result of the histochemical potassium reaction. The scarcely noticeable diffuse pale yellow color is not the result of the histochemical reaction, but arises by simple staining.

For the control tests in the histochemical reaction for potassium, the sections are immersed for 1 hour into 10% solution of phospho-12-tungstic acid, or for longer periods into solutions of lower concentrations, thoroughly washed in distilled water, and subjected to the Carere-Comes reaction. The resultant preparations are compared with sections treated by the Carere-Comes procedure without treatment with phospho-12-tungstic acid.

The sections for histochemical tests for potassium must be prepared from freshly frozen pieces. Embedding in paraffin wax is permissible after preliminary freezing and vacuum drying. Fixation in 10% formalin followed by embedding in paraffin wax, as recommended by Carere-Comes, apparently reveals potassium which is bound with the cell proteins and which therefore does not dissolve while the material is in the fixing agent and alcohols.

With this method it is possible to carry out histochemical studies of the location of potassium in cells, and selective staining of elements rich in potassium, such as erythrocytes and other cells of the erythroblastic series. Great interest attaches to histochemical investigation of potassium in tumors, in which it is present in considerably larger amounts than in normal tissues.

In conclusion, it is my duty to express my deep gratitude to Prof. L. I. Falin, who drew my attention to the need for control experiments in histological tests for potassium.

SUMMARY

A simple method of preparing sodium paradipicrylamine patented by Carere-Comes under the name of "Sienna Orange" as a histochemical test for potassium is described. The following method of controlling the specificity of the histochemical reaction for potassium is offered: the preparation is treated phospho-12-tungstic acid which reacts on potassium contained in the section; therefore, potassium cannot be combined with sodium paradipicrylamine.

LITERATURE CITED

[1] P. V. Makarov, Physicochemical Properties of the Cell and Methods for Their Investigation* (Leningrad, 1948).

[2] E. A. Nikitina, Zhur. Obshchei Khimy, Vol. 5, No. 8, pp. 1133-1138 (1935).

[3] A. L. Shabadash, Histochemistry of Glycogen in the Normal Nervous System* (Moscow, 1949).

[4] O. Carere-Comes, Z. wiss. Mikroskop., Bd. 55, H. 1, S. 1-7 (1938).

[5] L. Lison, Histochimie animale methods et problems, (Paris, 1936).

[6] A. B. Macallum, Journ. Physiol. Vol. 32, pp. 95-128 (1905).

* In Russian.

QUANTITATIVE DETERMINATION OF ADENOSINE TRI- AND DIPHOSPHATES

I. F. Seits

From the Biochemical Laboratory of the Leningrad Institute of Blood Transfusion

(Director A. D. Belyakov)

(Received June 23, 1956. Presented by Full Member of the Acad. Med. Sci. USSR, Prof. D. N. Nasononov)

Quantitative determination of adenosine triphosphate is one of the commonest and most necessary methods of biochemical investigation in laboratory practice. In recent years it has also become necessary to determine the specific activity (Sp. A) of adenosine triphosphate (ATP) phosphorus, as a measure of the metabolic potential in the cell. Many investigations have involved the determination of easily hydrolyzable tissue phosphorus by simple hydrolysis in 1 N HCl at 100° for 7 or 10 minutes. The values found generally reflect the quantitative content of ATP fairly accurately, but such analysis frequently distorts the true fractional distribution of phosphorus, especially if other easily hydrolyzable compounds are present. Moreover, the simple hydrolysis method does not allow of the determination of the specific activity of the ATP phosphorus. Precipitation of ATP by mercury or chromatographic determination cannot always be used, and are not always sufficiently accurate. Enzymatic methods for determination of ATP are also not very convenient.

Thus, there is no sufficiently reliable and accurate method, suitable for a wide range of laboratories, for determination of adenosine triphosphate. In this connection it is necessary to note the principle of the quantitative method for determination of ATP, originally put forward by Fiske [3] and applied in practice by Crane and Lipmann [2]. The method is based on adsorption of nucleotides on activated carbon and subsequent elution of the adsorbed ATP, ADP, and adenylic acid by aqueous pyridine, or removal of ATP and ADP labile phosphorus from the carbon by hydrolysis in 1 N HCl for 10 minutes at 100°.

Analysis of tissues for ATP and ADP by adsorption on carbon has been used for 2 years in the Biochemical Laboratory of the Leningrad Institute of Blood Transfusion. During this period its specificity has been confirmed, the possibility of its use for analysis of complex mixture of phosphorus-containing compounds has been established, and the optimum analytical conditions have been determined. As the results of detailed tests of the method of determination of ATP and ADP by adsorption on carbon, it may be described as a simple, rapid, and exact method, accessible and suitable for a wide range of laboratories.

Ba salts of ATP prepared by the Szent-Gyorgyi method, and Na salt preparations produced by the Ivanovo Meat Combine, were used in the investigations. ADP was obtained by enzymatic hydrolysis of ATP (by myosin). Adenylic acid was prepared by Kerr's method [5], cozymase by the Williamson and Green method [7], fructose-6-phosphate and fructose-1,6-diphosphate by the method of Neuberg et al. [6], and glucose-1-phosphate by the method of Sumner and Somers [1].

The most important factors influencing the results of quantitative determinations of ATP and ADP by adsorption on carbon are the pH of the medium during adsorption, the relative amount of adsorbent, and the procedure for the subsequent removal of extraneous impurities from the carbon with the adsorbed nucleotides. The optimum acidity for quantitative adsorption of ATP and ADP is pH 0.4-0.6. Accordingly, the best concentration of trichloroacetic acid in experiments with trichloroacetic acid centrifugates is 4-5%. Table 1 gives data on the influence of acidity of the medium on the degree of adsorption of pure ATP and ADP by carbon.

Some deviation from the optimum acidity, especially a decrease (down to 1%) has no significant influence on the results. Even in a neutral medium up to 70% ATP is adsorbed. This relative independence of adsorption on the pH of the medium is especially important if the experimental conditions are such that strong acidification must be avoided.

TABLE 1

Effect of Acidity of the Medium on the Degree of Adsorption

Substance	γ of labile P from data for 10-min hydrolysis in 1N HCl at 100°	Found			γ of phosphorus at a final trichloroacetic acid concentration (%)			
		0	1	4	4.5	5	8	9
ATP	185	128	—	—	180	—	—	140
ATP	125	—	115	119	—	—	100	—
ATP	60	—	—	—	55	—	—	45
ATP	50	—	44	47	47	46	—	—
ADP	50	—	—	45	45	—	—	—
ADP	50	—	—	45	—	46	—	—

The relative proportions of the amounts of carbon and adsorbed material are very important in relation to the accuracy of determinations of ATP and ADP. With the ordinary domestic specimens of active carbon (AG-2 and others) practically complete adsorption of ATP can be obtained by taking 1 mg of carbon per 1γ of easily hydrolyzable phosphorus. Since the amount of ATP in various cells and tissues rarely exceeds 200-300 γ of labile phosphorus per 1 g raw weight, 200-250 mg of carbon should be taken per each gram of cells or tissues. Table 2 gives the results of one of the experiments illustrating the effect of the quantitative proportions of the adsorbent and adsorbate on the degree of adsorption.

TABLE 2

Effect of the Proportions of Adsorbent and Adsorbate on the Degree of Adsorption

Carbon taken, in mg	ATP taken, in γ of phosphorus after 10-minute hydrolysis	Labile phosphorus found by the adsorption method	
		γ	% yield
100	45	43	95.5
100	90	87	96.4
100	180	165	90.2
100	360	270	75.0
100	540	350	64.8

Tests for specificity of the adsorption provided a very important criterion of the value of the method. The possibility of adsorption by the carbon from solution of other phosphorus compounds, capable of liberating orthophosphate after 10 minutes of hydrolysis in 1 N HCl at 100° was first examined. It was found that glucose-1-phosphate, fructose-1,6-diphosphate, and inorganic pyrophosphate are not adsorbed to any practical extent by carbon in the experimental conditions, and do not influence the yield of ATP. Diphosphopyridine nucleotide (DPN) is adsorbed by carbon. Our DPN preparation (≈ 60% pure) contained 17 γ of phosphorus, split off by 10 minutes of hydrolysis in 1 N HCl at 100°, per 1 mg dry weight. Carbon treatment gave 13.8 γ of 10-minute phosphorus per 1 mg DPN. However, the amounts of DPN in cells are small; for example, erythrocytes of the rabbit contain about 100γ per ml [4]. It is therefore evident that adsorption of DPN together with adenine

nucleotides by the carbon should not have any practical effect on the determination of ATP and ADP. Treatment of 1 ml of erythrocytes, containing 0.1 mg DPN, with carbon, would liberate little more than 1 γ of phosphorus.

An advantage of the method for isolation and determination of ATP by adsorption on carbon is the ease with which inorganic phosphorus can be removed, especially in experiments with radioactive phosphorus, from carbon containing adsorbed ATP and ADP. At P^{32} concentrations up to 0.5 μC per 1 ml of experimental mixture, twofold washing with water is enough to remove inorganic phosphorus from carbon containing ATP and ADP. With higher activities and in certain special cases it is necessary, before the washing with water, to wash the carbon once or twice with a mixture (1:1) of 0.05 M KH_2PO_4 solution and trichloroacetic acid solution. In one experiment 5 ml of a mixture containing nonradioactive ATP, corresponding to 200 γ of labile phosphorus, and 500 γ of orthophosphate with an activity of 1.0 μC was treated. After the usual procedure, 190 γ of easily hydrolyzable phosphorus was found on the carbon. The inorganic phosphorus was practically not determined, and the radioactivity of samples obtained by 10-minute hydrolysis of the carbon in 1 N HCl at 100° was 300 pulses calculated for the whole hydrolyzate (i.e., the whole original sample).

The possibility of determining ATP in complex mixtures of phosphorus compounds was also examined. A definite quantity of ATP was added to a mixture of orthophosphate, inorganic pyrophosphate, glucose-1-phosphate, fructose-1,6-diphosphate, fructose-6-phosphate, DPN, and adenylic acid (Table 3).

TABLE 3
Determination of ATP in a Mixture of Phosphorus Compounds

Composition of mixture in γ of phosphorus*		Increase in orthophosphate after 10 minutes of hydrolysis in 1N HCl at 100°, γ	Amount of easily hydrolyzable phosphorus
Inorganic orthophosphate	200	122	6
Inorganic pyrophosphate	50		
Glucose-1-phosphate	50		
Fructose-6-phosphate	50		
Fructose-1,6-diphosphate	50		
DPN	18		
Adenylic acid	20		
Ditto + ATP	180	302	175

* In γ of easily hydrolyzable phosphorus for ATP, glucose-1-phosphate, and pyrophosphate.

Table 3 shows that ATP is effectively differentiated from the other easily hydrolyzable phosphorus compounds.

The following standard procedure for analysis of cells and tissues for ATP, ADP, and inorganic phosphorus may be recommended. 3-4 ml of trichloroacetic acid centrifugate at a trichloroacetic acid concentration of 4-5%, and containing a quantity of cells corresponding to 1 g raw weight, is placed in the centrifuge tube with 250 mg of finely ground activated carbon (a weighing inaccuracy of 10-20 mg is permissible) and the mixture is thoroughly stirred with a glass rod. The suspension is left for 10 minutes with periodic stirring. For better sedimentation, 0.2 ml of alcohol (redistilled) is poured in a layer on top of the mixture, and the carbon particles which rise up the moist walls of the tube are carefully pushed into the alcohol layer with the rod. The suspension is centrifuged at 2000-3000 rpm for 10-15 minutes, and the liquid (liquid A) is decanted off. The carbon with the adsorbed ATP and ADP is washed twice with 5 ml of distilled water. The precipitate is carefully

stirred in the water each time by a rod, and a layer of 0.2 ml of alcohol is applied before centrifuging. The wash liquids are rejected. In special cases the carbon is washed with the mixture described above before the water washing. An equal volume of 2N HCl and 2-3 ml of 1N HCl is added to the carbon, and the samples are hydrolyzed for 10 minutes at 100°. The labile phosphorus of ATP and ADP is split off by hydrolysis and passes into solution. After hydrolysis, the samples are brought up to their original volume with water, or their new volume is noted. The samples are centrifuged this time without addition of alcohol, and the liquid is taken for photometric and radioactivity determinations. In parallel with the experimental samples, a portion of carbon is treated with the corresponding amount of 4 or 5% solution of trichloroacetic acid (according to the concentration of acid in the test samples). This sample is used as the reactive sample in experiments with the universal photometer or photoelectrocolorimeter.

The liquid A contains other organic phosphorus compounds, in addition to orthophosphate. Mineral phosphorus is precipitated in it by the usual methods. The precipitate contains orthophosphate, and the liquid contains hexose and triose phosphates and other organic phosphorus compounds. Since the inorganic phosphorus is precipitated in a solution already free from ATP and ADP, contamination of the precipitate is slight and in a number of cases reprecipitation is not necessary.

Adsorption of ATP and ADP on carbon may serve as the basis for a relatively simple, rapid, and fairly accurate method for determination of the contents and specific radioactivities of these compounds.

SUMMARY

The specificity and conditions of the application of the determination method of adenosine tri- and adenosine diphosphates by means of nucleotides adsorption on coal (method developed by Crane and Lipmann) have been studied. It has been stated that the presence in samples, besides ATP and ADP, of fructose-1, 6-diphosphate, fructose-6-phosphate, glucose-1-phosphate, inorganic pyrophosphate, orthophosphate, adenylic acid and DPN does not interfere with the determination of ATP and ADP. Completeness of ATP adsorption depends upon the pH of the medium, as well as upon the ratio between the adsorbed substance and the adsorbent. Acidity corresponding to 4-5% trichloracetic acid and the ratio 100 mg of coal per 100 γ of readily hydrolyzed phosphorus of ATP are the most favorable conditions. This method is simple, specific, and accurate.

LITERATURE CITED

[1] J. B. Sumner and G. F. Somers, Chemistry and Methods of Enzymes,* Moscow, 1948.

[2] R. K. Crane and F. Lipmann, J. Biol. Chem.v. 201,pp. 245-246 (1953).

[3] C. H. Fiske, Proc. Nat. Acad. Sci., v. 20,pp. 25-27 (1934).

[4] E. C. G. Hofmann, Biochem. Ztschr., Bd. 327, N. 4, S. 273-283 (1955).

[5] S. Kerr, J. Biol. Chem., vol. 139, pp. 131-134 (1941).

[6] C. Neuberg, H. Lustig, and M. A. Rothenberg, Arch. Biochem., v. 3,pp.33-44 (1943).

[7] S. Williamson and D. E. Green, J. Biol. Chem., v. 135,pp.345-346 (1940).

* In Russian.

USE OF RESISTANCE PICKUP UNITS FOR RECORDING GASTROINTESTINAL MOVEMENTS

T. D. Dzudziguri

Laboratory of corticovisceral pathology (Director: Prof. I. T. Kurtsin)
I. P. Pavlov Institute of Physiology (Director: Academician K. M. Bykov,
Academy of Sciences of the USSR, Leningrad

(Received November 6, 1956. Presented by Academician K. M. Bykov)

The present paper describes the use of resistance pickup units for recording gastrointestinal movements in series experiments on dogs.

The units were thin elastic rubber tubes 1-2 cm long and 1-1.5 mm in diameter tightly packed with microphone carbon powder. The carbon was in contact with the cleaned ends of flexible wires, PVC*-insulated. The outgoing ends of the wires passed through a housing (best made of "Lucite") and were soldered into the pins of a tube base fixed at the remote end of the housing (Fig. 1).

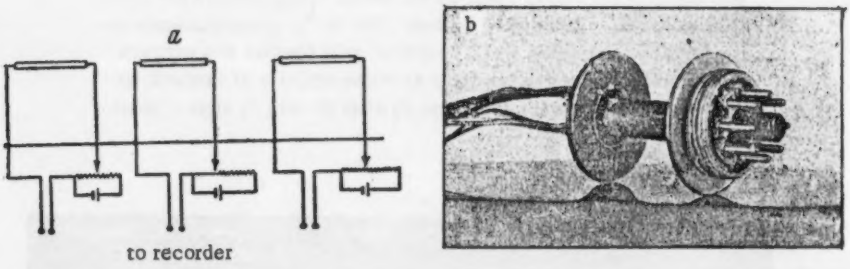


Fig. 1. a) Wiring circuit of carbon pickups and, b) the plastic housing with tube base used to bring the leads out from the peritoneal cavity.

The peritoneum was opened under general anaesthesia and the sterile pickups attached to the selected part of stomach or intestine with knotted bandages. The outgoing leads, together with part of the housing remaining inside the peritoneum, were coated with grease and fixed to the peritoneal incision. Damage to the base pins was avoided by covering them with a metal cap. The dogs withstood the operation well. This method of bringing out internal leads was devised by A. D. Golovsky, of the Department of Normal Physiology, Kirov Academy of Military Medicine.

A low DC current was passed through the pickup during the postoperative period; the pickup resistance changed with the movement of the section to which the unit was fixed; recordings were made on a recording oscillograph.

The movements of stomach, intestine and gall bladder were recorded during periodic movement and under food stimuli. Several areas of digestive tract were found to move together in periodic movements; as the duration

* polyvinyl chloride

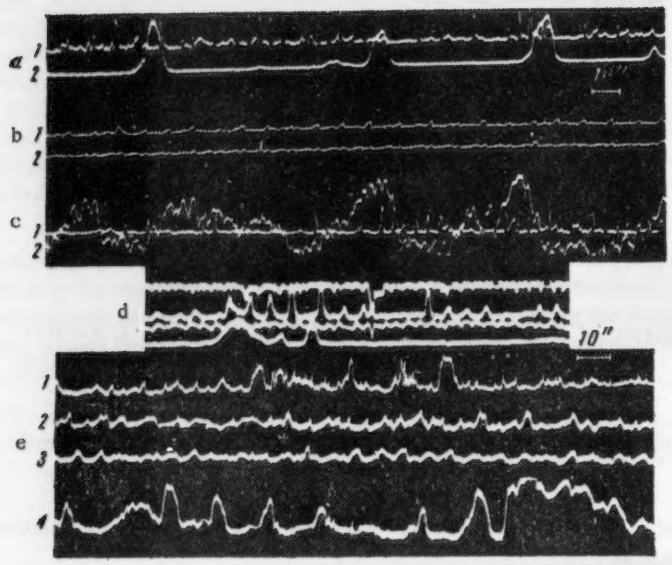


Fig. 2. The motor activities of various gastrointestinal areas recorded with the transducers.
a) Simultaneous recordings of contractions in the 12-finger gut (1) and stomach (2) during work; b) the same, during rest; c) the same in response to inflation of a balloon (250 cm^3); d) simultaneous recordings of respiratory, small intestine, gall bladder and stomach movement (from top to bottom); e) motor activity of stomach on feeding 250 g of bread: 1) before; 2) after 30 min 3) after 2 hours, 4) after 4 hours.

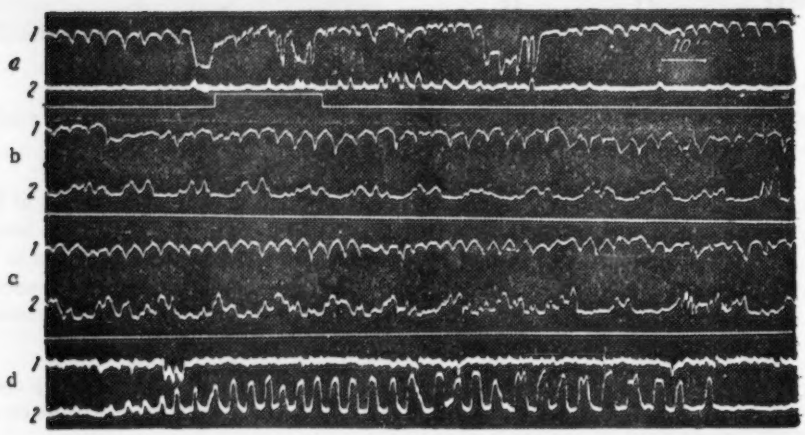


Fig. 3. Activity of gall bladder on eating 100 g of meat.
a) Instant of ingestion; b) after 1 hour; c) after 2 hours; d) small intestine activity; 1) respiration; 2) intestinal contractions.

of rest increased the movements gradually became weaker and ceased. Food caused small local contractions which became stronger over 4-5 hours, resembling contractions occurring during work (Fig. 2).

The units were also used to record movements in the isolated duodenum (Fig. 2,d). The pickup was attached to a drainage tube inserted in the duodenal cavity. The secretory and motor activities can be studied during the whole digestion process in this way.

As the units are small they do not stimulate the mechanoreceptors, so the continuous rhythmic contractions observed by normal balloon methods are absent.

When the movements of stomach and isolated duodenum were recorded together it was found that they were strongly correlated. No contractions occurred in the latter at rest. The movements in both organs begin together.

Food initiates small local contractions which persist during secretion, and as secretion falls off and the gastric juice becomes less acid the contractions become stronger, passing over to periodic infrequent strong contractions, rest supervening after a definite interval.

Figure 3 shows a record of gall bladder activity.

The activities of gall bladder and intestine can be recorded without fixing the pickup by inserting it through a fistula tube. Here also the small size and high sensitivity facilitate recordings during digestion without stimulating the mechanoreceptors in the gall bladder.

SUMMARY

Carbon pickup units for registration of motor activity from different portions of the digestive tract and of the gall-bladder under different conditions of series experimentation are described. Units are implanted in the areas under study, and output terminals are soldered to the pairs of a plastic coil which is fastened in the abdominal wall.

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE IN ENGLISH TRANSLATION

February, 1957

TABLE OF CONTENTS

	Page	Russ. Page
1. The Dominant Focus of Excitation in the Formation of a Conditioned Reflex. M. Ya. Rabinovich and L. G. Trofimov	129	3
2. Iono-Humoral Interrelations in the Process of Appearance and Development of Vagal Inhibition of the Heart. Communication I. The Influence of Atropine on the Normal and Desympathized Frog Heart. I. A. Keder-Stepanova	134	8
3. Cerebral Circulation Under Reflex Influences. Communication I. Cerebral Blood Supply on Stimulation of Small Intestine Interoceptors. N. M. Ryzhova	139	13
4. Gastric Mechanoreceptor Reflexes Before and After Hemidecortication. S. S. Musyashchikova	143	18
5. The Role of the Pancreas in the Complex Reflex Regulation of Carbohydrate Metabolism. I. S. Kanfor	148	22
6. The Sweating Reactions of the Palm. O. A. Krylov and E. G. Deryugina	152	26
PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY		
7. Changes in Erythrocyte Count and Hemoglobin Content in the Peripheral Blood Under Prolonged Nociceptive Irritation. V. V. Kravtsov	156	30
8. The Influence of Removal of the Cerebral Cortex and Certain Pharmacological Substances on the Development and Course of Fever. L. I. Gorbatsevich	161	35
9. The Sequence of the Disappearance of Oculomotor Reflexes in Traumatic Shock. V. D. Lindenbraten	166	39
BIOCHEMISTRY AND BIOPHYSICS		
10. The Half-Life of the Different Serum Protein Fractions in the Circulating Blood. M. S. Surovikina	172	45
11. The Metabolism of Hydrogen Sulfide (H_2S^{35}) Injected Subcutaneously. A. I. Gunina	176	48
12. Temperature Changes of the Human Skin Irradiated with Low Intensity Waves Several Centimeters in Length. A. S. Pressman	180	51
13. The Distribution of Free Sulfhydryl Groups in the Proteins of the Mucous Membrane of Different Divisions of the Stomach and the Change in the Amount of Them in Connection with the Secretion of Gastric Juice. E. E. Martinson and H. P. Lind	185	55
PHARMACOLOGY		
14. The Ganglioplegic Properties of Aminazine (Largactil) and Mepazine (Pacatal). D. A. Kharkevich	188	58
15. The Fate of Syntomycine and its Optical Components in Animal Organisms. G. N. Pershin and V. V. Nesvadba	191	61

TABLE OF CONTENTS (continued)

	Page	Russ. Page
MICROBIOLOGY AND IMMUNITY		
16. Relation Between the Level of Specific Antitoxin in the Blood and the Degree of Immunity to Tetanus Toxin in White Mice Immunized with Adsorbed Tetanus Toxoid. A. A. Vorobyev, B. S. Goncharov, L. P. Lubyansky, and A. V. Markovich	193	63
17. Change in the Antigenic Composition of Certain Parenchymatous Organs in White Mice Infected with the Agent of Dysentery and Streptococci. M. V. Zemskov	197	66
18. Experimental Biological Model of Bacillary Dysentery. V. V. Budylina, A. Yu. Illyutovich, Z. S. Petrova, T. V. Bodulina, E. E. Golubeva, A. I. Titrova and R. S. Chetvernina	201	70
EXPERIMENTAL BIOLOGY		
19. Cell Division and Vitamin A. I. A. Alov	206	76
20. The Role of the Thyroid Gland in the Regulation of Cell Division. I. E. Epova	210	80
21. On the Causes of Regeneration Ability Loss in the Axolotl Extremity After X-Raying. V. F. Sidorova	215	84
22. The Antibiotic Properties of the Ejaculates of Mammals and Man. V. N. Kulikova	220	89
23. The Functional Significance of the Innervation of the Human Umbilical Cord. V. N. Blyumkin	223	91
24. The Content of Acetylcholine-Like Substances and Cholinesterase in the Tissues of the Ascaris. A. I. Krotov	227	95
MORPHOLOGY AND PATHOMORPHOLOGY		
25. Change in the Transitional Epithelium in the Case of Reparative Regeneration. M. P. Ptokhov	230	98
26. Duration of the Effect of Medicamental Sleep on Inflammation. E. D. Klimenko	235	101
27. Changes in the Peripheral Nervous System Due to Experimental Fever. V. V. Astakhova	241	105
28. The Condition of the Vascular-Capillary System of the Brain in Animals of Different Ages During Asphyxia. Article II. T. P. Zhukova	246	109
29. Changes in the Organs of White Rats Due to an Intraperitoneal Injection of Quartz Dust. G. V. Belobragina	251	114
NEW METHODS		
30. Method for the Histometric Determination of Potassium. M. G. Shubich	255	118
31. Quantitative Determination of Adenosine Tri- and Diphosphates. I. F. Seits	257	119
32. Use of Resistance Pickup Units for Recording Gastrointestinal Movements. T. D. Dzudziguri	261	123

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO.	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

E A·I·B·S bulletin

ficial
blication

NEWS AND VIEWS IN THE BIOLOGICAL SCIENCES

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES
0 P Street, N.W., Washington 6, D.C.
ms 4-5573

If you are a biologist....

If you are interested in the biological sciences....

The AIBS BULLETIN is a "must".

This magazine offers news and information important to every biologist. It carries book reviews, brief and pertinent, on all new publications in the field. Feature articles deal with all manner of general and controversial topics in biology. The advertisers are specialists in products needed by every teacher and research worker. The August issue is a special General Program for the annual meeting of biological societies that the Institute sponsors.

The AIBS BULLETIN may be obtained in any one of the following ways.

1. Through your membership in one of the nineteen Member Societies of the Institute, you automatically receive the BULLETIN free of charge, as part of your membership dues.
2. If you belong to one of the eighteen Affiliate Societies of the Institute, you may subscribe to the BULLETIN for $1.00 per year.
3. Regular subscription prices for non-members are: U.S.A., $2.00 per year; Canada, South and Central America, $2.50; all other foreign, $3.00.
4. Various categories of Individual Membership are offered by A.I.B.S.: Associate, $1.00; Professional, $3.00; Supporting, $10.00; and Life, $100.00. A subscription to the BULLETIN accompanies each type of membership.

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES
2000 P Street, N.W., Washington 6, D. C.

E
UES
RLY

THOUSANDS OF JOURNALS

throughout the world are publishing significant research papers in

Experimental Biology and Medicine

any one of which might be of vital interest to you

How do you keep posted on current advances in your field? No individual possibly could read it all in the original even if the journals were available to him. *Biological Abstracts* does this arduous task for you. Currently publishing more than 40,000 abstracts of research papers in biology, it plans gradually to expand its coverage to 80,000 within the next ten years in order to keep pace with the ever-increasing output of biological literature.

Biological Abstracts, a cooperative, non-profit undertaking, is published by biologists for biologists in a complete edition covering all fields of biology, and five low-priced sectional editions that are specially designed for individual use. Two of these sectional editions have particular value for those working in Experimental Biology and Medicine:

SECTION B—each month 1,200 to 1,500 abstracts of research papers in Animal Physiology, Pharmacology, Morphology and Pathology, exclusive of Microbiology, are published.

SECTION C—each month some 450 to 550 research papers in Microbiology, including General Bacteriology, Medical Bacteriology, Viruses, Pathogenic Fungi, Chemotherapy, Immunology, Public Health, Industrial Microbiology, and Parasitology are abstracted.

Individuals are allowed a discount of 50% off the list prices of the sectional editions if the subscriptions are for their personal use.

Sample copies and complete information will be sent upon request.

BIOLOGICAL ABSTRACTS, University of Pennsylvania
3815 Walnut Street, Philadelphia 4, Pa.